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RESEARCH PAPER RP1597

Part of Journal of Research of the National Bureau of Standards, Volume 33, August 1944

INTERFEROMETER MEASUREMENTS ON THE EXPANSION OF IRON

By James B. Saunders

ABSTRACT

The interferometer has been applied by many investigators to the measurement of thermal expansion but has failed to yield the precision that they apparently expected and sometimes have claimed. In their explanation of the resultant discrepancies, most observers have attributed them to actual differences in the physical properties of the sample; however, some have admitted failure to find a satisfactory explanation.

Several sets of data, taken on relatively pure iron by different observers and different procedures, are compared. The results show good agreement between data taken with those interferometer methods that are free from tilting of spacers and air-film errors, whereas the failure to eliminate these two errors produces data that cannot be duplicated except by chance. The interferometer data that are free from these errors also agree satisfactorily with data that have been obtained by other precision methods.

Some investigators claim to have found indications of a characteristic temperature effect in the expansivity curve of iron in the temperature range from 0° to 250°C. It is shown that when the expansion data are freed from errors of tilting and changes in air films, the indications of such effects do not appear.

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I. INTRODUCTION

Many observers have measured the expansion of relatively pure iron. Several different methods of measurement have been used, among which that employing the Fizeau [1]* type of interferometer, in the form described by Peters [2] and Merritt [3], has been considered [4] extremely accurate. However, as will be shown later, the data taken by different observers, with this particular form of interferometer, do not always agree. Often data obtained on different samples of the same material by a single observer have shown differences far in excess of the precision that should be attainable with this instrument.

Considerable stress has been placed on the presence of certain reversals in curvature which have been found in the expansivity curve of iron in the range 0° to 400° C. In some cases these effects appear to be mainly the result of inadequate precision. That is, these reversals in curvature occur at different temperatures in data obtained

*Figures in brackets indicate the literature references at the end of this paper.

on similar samples by different observers and also in data obtained by a given observer on different samples. This indicates that they are not a definite characteristic of relatively pure iron. This indication is supported by the results of Driesen [5], which show that the reasonably definite irregularities in the expansion of impure iron in this temperature range practically disappear when the carbon content is less than 0.1 percent. However, some later results obtained by the interferometric method have been interpreted as showing that an irregularity still appears in this temperature range even when the carbon content is less than 0.01 percent.

This paper gives the results of additional measurements of the expansion of high-purity iron. These measurements were made by the Fizeau interferometer method with a procedure which substantially eliminates errors caused by a tilting of the spacers and by variation in the thickness of air films between the spacers and interferometer plates at their points of contact.

II. SOME PREVIOUS INTERFEROMETRIC RESULTS ON IRON

Of those who have investigated the expansion of iron by the interferometric method, Dorsey, Austin and Pierce, Adenstedt, and Nix and MacNair have obtained results that will be considered here.

In 1907 Dorsey [6] measured the expansion of relatively pure iron with an interferometer arrangement using a vacuum furnace and the ring type of sample proposed by Reimerdes [7]. Data taken with this type of sample, in vacuum, are practically free from both air-film errors and tilting of spacers caused by differential expansion. Having eliminated both tilting and air-film errors by this method, Dorsey then considered the sources of error that the assumed precision of the measurements indicate as possibly inherent in the method. The indicated errors were found to be relatively small, but concerning the expansion data he adds this illuminating statement, "individual intervals for some specimens, however, show differences among themselves far in excess of the above-indicated errors." However, Dorsey's data are in fair agreement with data obtained by others who employed equally precise methods (see fig. 4).

In 1934 Austin and Pierce [8] published data on 10 samples of relatively pure iron. The method used was that described by Merritt and subsequently [9] found to be subject to the previously mentioned errors. The expansivities obtained for the 10 samples differed among themselves in the range 0° and 400° C. The differences from sample to sample were attributed by Austin and Pierce to small differences in impurities. However, these differences in expansivity, and the irregularities also, do not appear to the author to exceed the possible lack of precision of the measuring method employed.

In 1936 Adenstedt [10], while using practically the same interferometer arrangement that Austin and Pierce used, measured the expansivity of iron in a wide range below 0°C and observed that the width and orientation of the interference bands did not remain constant as the temperature changed. Consequently, he discarded all tests (15 out of 36) in which this effect, caused by a tilting of the samples, was observed. As a result of this experience, Adenstedt devised an ingenious arrangement for supporting his samples and

binding them together in an essentially ring-like spacer that eliminated tilting. This appears to have improved the precision considerably beyond that resulting from the rather questionable expedient of discarding the apparently less desirable tests. Unfortunately, he did not use this device in testing iron.

In 1941 Nix and MacNair [4] also used the interferometer method as employed by Austin and Pierce except that they replaced visual observation by a photographic method for recording the shift of the interference fringes. The data which they published for iron were procured by testing three samples obtained from the National Bureau of Standards. The total impurities of these samples varied from approximately 0.008 to 0.012 percent. Concerning the differences in the results on these samples, Nix and MacNair stated that the "variations from sample to sample are quite considerable in the region above room temperature where presumably the effects of extremely small amounts of impurities can materially affect the ferromagnetic change in length." As computed, their results for the expansivities resemble somewhat those obtained by Austin and Pierce. That is, these results indicate a plateau-like effect in some of the expansion curves near the rather low temperature of 140°C.

Before the measurements discussed in the following pages were made, the expansion of other samples of the particular iron studied by Nix and MacNair was also measured by others, including the present author. In none of these cases was the plateau-like effect observed but, in the range 25° to 150°C, the results of the present author were lower than those of Hidnert and Emerson as shown in figure 9 of the publication by Cleaves and Hiegel [11]. It was believed that this discrepancy in the author's data, which reached a maximum of about 3 percent of the expansivity at 25°C, was the result of small temperature gradients that developed (on heating at a rate of 1 degree C per minute) between the refraction thermometer and the rather massive specimens of iron that were used. In procuring these data the interest centered primarily in eliminating erratic errors that might cause spurious plateau-like effects rather than in carefully avoiding all small errors that might appear because of slight gradients. Such gradients develop rapidly on starting and then slowly diminish as the heating rate rises from zero to the constant value chosen. Any material discontinuity in the heating rate, during a test, will produce such changes in the thermal gradients and often gives rise to corresponding irregularities in expansion data. That is, to attain consistent results and to prevent spurious irregularities, a uniform heating rate should be maintained. Also any adjustments that change the heat conduction within the specimen chamber or that permit unsymmetric radiation from it should be avoided if possible.

III. COMPOSITION AND TYPE OF SAMPLES TESTED

In the present investigation, the expansivities of several samples from the high-purity iron that yielded the results presented by Nix and MacNair and that was previously tested at this Bureau were measured between 25° and 260°C. No significant differences from sample to sample were found. To study the possible effects of differences in the amounts of impurities in relatively pure iron, tests were also made on samples from an electrolytic iron and from an

open-hearth ingot iron. (The impurities of these irons are shown in table 1.) For this comparative study of the three irons, the tests were extended to 400°C.

TABLE 1.—*Composition of samples*

Elements	Samples			Elements	Samples		
	A	B	C		A	B	C
C	0.001	0.005	0.045	Cr		<.001	.00
Cu	<.002	<.001	.046	Mn		.002	.04
Si	.001	.001	.001	Ni		<.001	.01
Be	<.001			Mo			.00
Al	nil	<.001	.002	Co			.00
S	.002[3]	<.004	.03	As			.01
P	<.0005	<.003	.003	Sn			.00
O_2	.000[3]	.003	.07				
N_2	.000[2]	.002	.004	Total of identified impurities	<0.008	<0.024	<0.30
H_2	.000[2]	.000[1]					

In some of these later tests, three individual tripod spacers were used as in the previously mentioned measurements, but they were no longer of the massive type. That is, as a possible aid in reducing thermal gradients, the weight of the spacers was reduced to one-third by cutting away much of the unnecessary portions. In the remainder of the tests, a single T-shaped spacer was used, and its weight seldom exceeded 1.5 grams, although its height usually exceeded a centimeter. As a further possible aid in reducing gradients, the shape of the specimen was designed, whenever possible, to facilitate the transfer of heat between it and the surrounding gas. These steps are advisable because the rate of heat transfer through the point contacts between plate and spacer is low.

IV. METHOD OF MEASUREMENT

Most of the following data were obtained visually by means of the usual viewing instrument. However, in some cases a new type of photographic recorder [1] was used. Sample records are reproduced in figure 1 to show the small amount of film required. The recorder was attached directly to the viewing instrument without disturbing the interferometer in the furnace, when the change from visual to photographic recording was made.

In assembling the interferometer, the weighted spacer method (see reference [9]) was used, and the reference point was located directly above the contact between this spacer and the upper plate. To stabilize the air films between this spacer and the interferometer plates, the assembly was always given a preliminary heating to 150° C and allowed to cool to room temperature before the first run for procuring data. The tests were made in air at atmospheric

[1] This type of recorder yields an accurate and continuous record of the changes in the interference fringes. The interferometer itself may be observed during the exposure without interfering in any way with the recording, and a surprisingly small amount of film is needed. One foot of 35-mm film is sufficient to record a shift of more than a hundred fringes. The recorder (designated an interferograph) produces the record (called an interferogram) in somewhat the same way that a panoramic camera operates. An image of the interferometer fringes is projected into the plane of the photographic film. A screen, located close to and in from of the film, having a slit similar to that of a focal-plane shutter, obstructs all of the image except a narrow strip. The film is caused to move perpendicular to the slit. The resultant exposure produces a record of the fringe movements relative to any point whose image falls in the exposing slit. This photographic recorder will be described in a later publication.

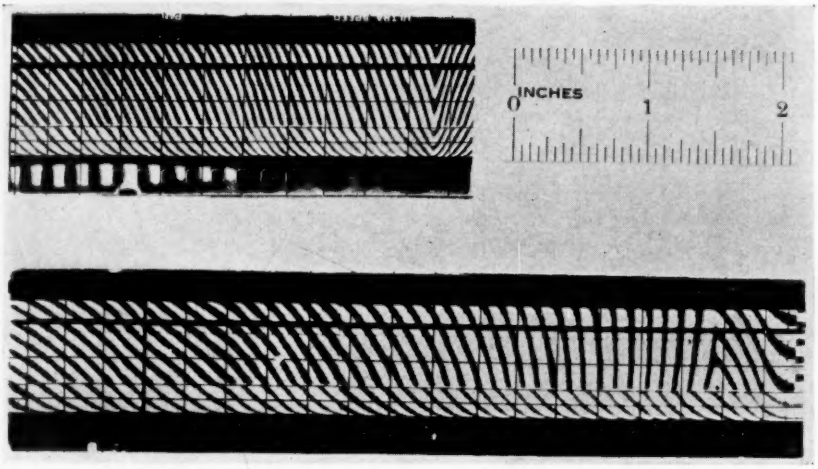

FIGURE 1.—*Two photographic records (interferograms) of both temperature and expansion of iron from room temperature to 260° C.*

The heating rate for the upper record was 1 degree C per minute and for the lower record, it was one-half degree per minute. The same difference in length of record, for a given temperature interval, is obtained by using the same heating rate and two different rates of film motion.

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pressure and no effects resulting from oxidation were noted.[2] The usual heating rate was approximately 1 degree C per minute except for the second photographic record, in which the rate was one-half degree per minute. Observations were made at the passage of each fringe in the thermometer plate.

V. RESULTS

Figure 2, A, shows a plot of the expansion curves obtained on one of the samples of the high-purity iron. The data of the first run were obtained visually. Those for the second and third runs were obtained photographically without disturbing the interferometer. In order to record the fringe shifts, both at the reference point over the weighted spacer[3] and at the median point (as defined in reference [9]), both points were included in the record. The tilting of the unweighted spacers is therefore made quite evident.

The plot of the observations (fig. 2, A), which were made at intervals averaging about 7 degree C, show excellent agreement when they were made at the weighted spacer. Also, there was no material difference between the visual and photographic data. As was to be expected, the data at the median point deviate considerably from those at the weighted spacer.

To make the discrepancies between these data more apparent, a type of residual plot that makes their magnification possible is shown in figure 2, B. To produce this figure, the ordinates of the straight line, $y=0.13T-1.50$, were subtracted from the corresponding ordinates of the expansivities plotted at different values of the temperature, T. As before, the agreement between the weighted spacer data appears to be excellent, but the deviation of the results at the median point from those at the weighted spacer point are shown to be very large except near room temperature, where the expansion curves were made to intersect. For comparison, that part of Nix and MacNair's results that was obtained in this range and on the same iron is also plotted in both figures here.

This departure of the curve, for the median point, from the curve for the weighted spacer point (as the temperature increases) is typical of the method. Also the author's curve for the median point is typical of the condition in which the spacers are all untilted as the heating begins, but in which at least one of the spacers develops a continually growing tilt as the temperature rises. At least a part of the median-point curve would have fallen below the weighted spacer curve had there been, at room temperature, an initial tilt of the kind that is caused by cooling the interferometer from some higher temperature at which the spacers have been brought into an untilted condition. That is, if this higher temperature had been above the temperature attained in these tests, the median-point curve would have fallen further and further below the weighted spacer curve as the temperature rose. However, if this temperature had been between the highest and lowest temperatures of the tests, this departure would have risen to a maximum and then decreased.

[2] Prolonged observation at a constant temperature of 400° C showed no progressive change in readings.

[3] Data recorded at the median point, on the second photographic record, deviated much more than it did during the first recording; amounting to over 7 percent. This deviation was so large that the corresponding results are omitted from figure 2, as its inclusion would require too much space.

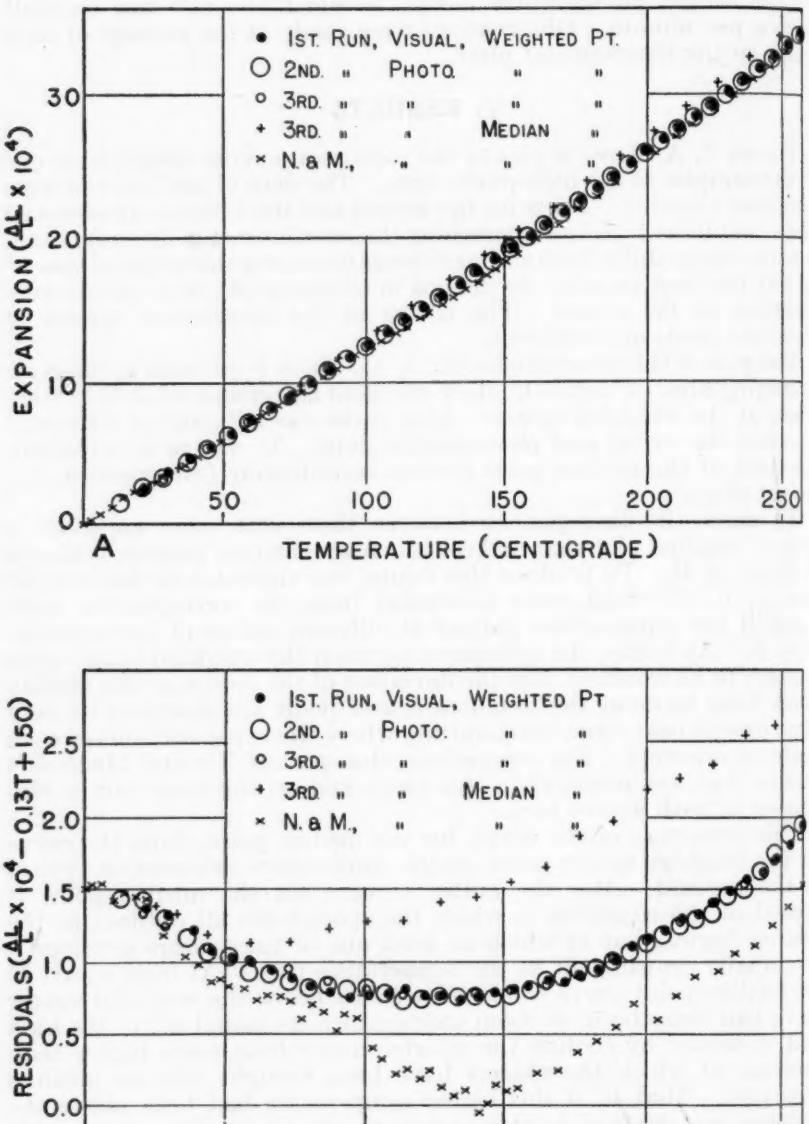


FIGURE 2.—*Linear thermal expansion of iron as determined by several interferometer procedures.*

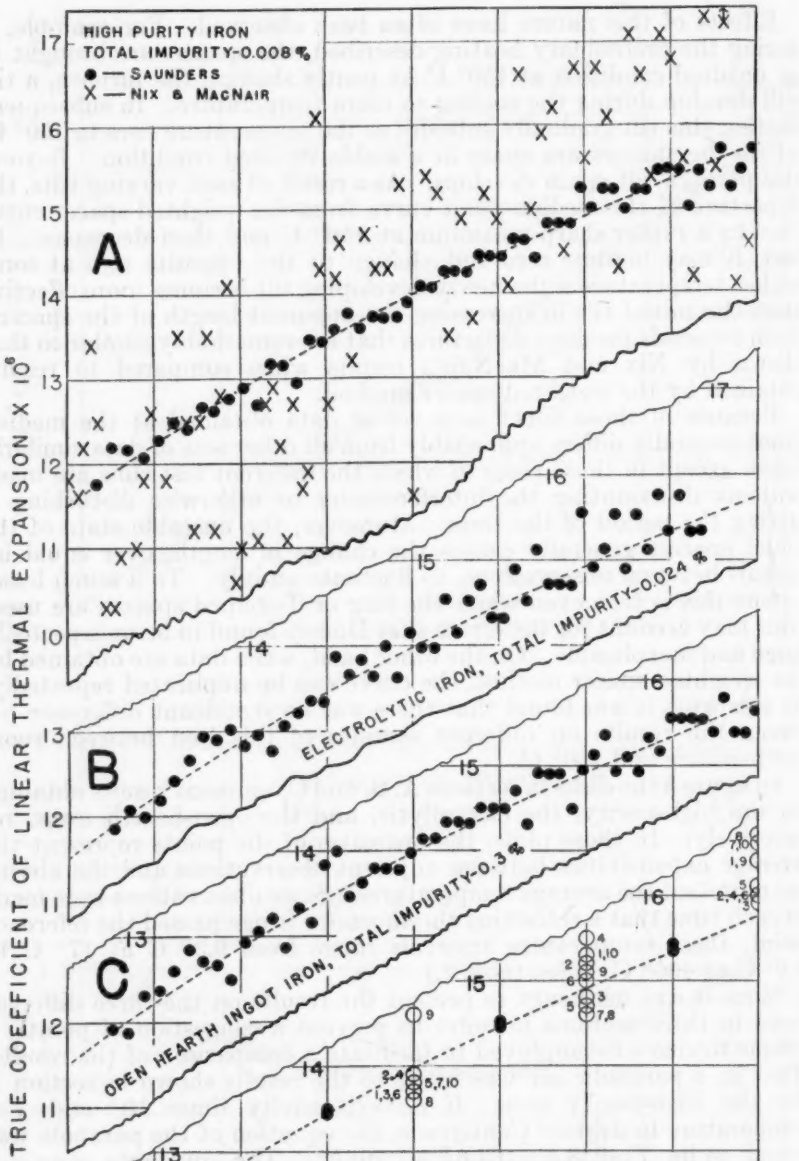
Effects of this nature have often been observed. For example, if during the preliminary heating described, the spacers are brought to an untilted condition at 150° C by gently shaking the furnace, a tilt will develop during the cooling to room temperature. In subsequent heating this tilt gradually subsides as the temperature rises to 150° C, where the spacers are again in a stable untilted condition. Beyond this point, a tilt again develops. As a result of such varying tilts, the departure of the median-point curve from the weighted spacer curve rises to a rather sharp maximum at 150° C and then decreases. In fact, it may become zero and change to the opposite sign at some higher temperature if the newly developing tilt becomes more effective than the initial tilt in increasing the apparent length of the spacers. Such reversals produce departures that are remarkably similar to that shown by Nix and MacNair's results when compared to results obtained by the weighted spacer method.

Because of these tilts [4] each set of data obtained at the median point generally differs appreciably from all other sets of data similarly taken except in those cases in which the different test runs are made without dismounting the interferometer or otherwise disturbing it during the period of the tests. Moreover, the unstable state of the tilted spacers generally causes the change in length, over equal intervals between observations, to fluctuate unduly. To a much lesser extent this is true even when the ring or T-shaped spacers are used. This may account for the errors that Dorsey found to be unexpectedly large and inexplicable. On the other hand, when data are obtained by the weighted spacer method, the curve can be duplicated repeatedly. In this way, it was found that there was no significant difference between the results on different samples of this iron between room temperature and 260° C.

In figure 3 the disks in sections A, B, and C represent results obtained on the high-purity, the electrolytic, and the open-hearth irons, respectively. In these plots, the ordinates of the points represent the average expansivities between adjacent observations and the abscissas represent the average temperatures. Since observations were made at each time that a refraction thermometer fringe passed the reference point, these temperature intervals range from 9.3° C at 17° C to 6.0° C at 400° C. (See table 2.)

Since it was necessary to present the results on the three different irons in three sections in order to prevent a congestion of points, a simple device was employed to facilitate a comparison of the results. That is, a parabolic arc was fitted to the results shown in section A for the high-purity iron. If $E \equiv$expansivity times 10^6 and $T \equiv$ temperature in degrees Centigrade, the equation of the parabola was found to be $T=238.6-104.6E+7.295E^2$. The constants were adjusted by the method of averages. The arc of this parabola is represented by a broken curve and is also introduced into the other sections of figure 3. In sections B and C it appears to fit the results of the corresponding samples almost as well as could be expected, even if all of them had been obtained on an identical iron.

[4] The author has taken data on many hundred samples of different materials and finds that seldom, if ever, does the top interferometer plate fail to show vagrant changes in its inclination with respect to the bottom plate.



Pt equals point, and N&M equals Nix and MacNair.

FIGURE 3.—*Comparisons of expansivity data for three samples of iron, having different amounts of total impurities, with some results already published.*

TABLE 2.—*Observed linear thermal expansion of relatively pure iron*

Temperature	Expansion in microns per centimeter			Temperature	Expansion in microns per centimeter		
	Sample A	Sample B	Sample C		Sample A	Sample B	Sample C
°C				°C			
12.4	0.00			229.4	28.40	28.18	28.34
21.7	1.10	0.99	0.98	236.2	29.34	29.16	29.31
30.8	2.21	2.06	2.08	242.9	30.33	30.14	30.28
39.9	3.31	3.14	3.18	249.6	31.30	31.10	31.25
48.8	4.38	4.20	4.27	256.3	32.26	32.06	32.22
57.5	5.45	5.26	5.34	262.9	33.22	33.03	33.18
66.0	6.52	6.31	6.40	269.5	34.19	33.99	34.13
74.3	7.55	7.35	7.46	276.0	35.13	34.97	35.10
82.4	8.58	8.39	8.49	282.5	36.09	35.93	36.07
90.5	9.60	9.43	9.52	289.0	37.05	36.88	37.03
98.5	10.63	10.47	10.53	295.5	38.02	37.83	37.98
106.4	11.64	11.47	11.53	301.8	38.98	38.82	38.92
114.2	12.63	12.47	12.55	308.2	39.95	39.77	39.89
121.9	13.64	13.45	13.56	314.5	40.91	40.72	40.85
129.6	14.66	14.45	14.55	320.8	41.86	41.69	41.79
137.2	15.64	15.46	15.57	327.2	42.82	42.64	42.75
144.7	16.65	16.45	16.55	333.5	43.79	43.62	43.69
152.0	17.63	17.43	17.57	339.8	44.72	44.57	44.65
159.2	18.61	18.42	18.57	346.0	45.69	45.55	45.60
166.5	19.62	19.41	19.55	352.2	46.65	46.50	46.56
173.7	20.59	20.40	20.53	358.4	47.60	47.47	47.52
180.8	21.57	21.37	21.51	364.6	48.55	48.45	48.48
187.9	22.54	22.35	22.48	370.8	49.51	49.39	49.44
195.0	23.54	23.32	23.47	376.9	50.45		50.39
202.0	24.55	24.31	24.45	383.0	51.41		51.35
208.9	25.49	25.27	25.42	389.1	52.34		52.28
215.8	26.45	26.23	26.39	395.2	53.28		53.22
222.6	27.42	27.21	27.36	401.2	54.22		54.15

A direct comparison of interferometer data that are taken with and without the weighted spacer prodecure is afforded when the data obtained on sample A and the data taken by Nix and MacNair, on a sample from the same iron, are treated similarly. These data are plotted in section A of figure 3. To make this comparison of the two sets of data on the same basis it is necessary to use approximately equal temperature intervals in both cases. Fortunately, this is easily possible, since Nix and MacNair made observations at intervals that were either approximately the same or about one-half as large as those used by the present author. Consequently, although approximately equal intervals were used always in the computations, their data are represented by double the number of points (X's) in the range below 150° C, in which they used the shorter intervals. The dispersions of the points resulting from this treatment of data may seem rather large, but it will be found not to be unusual if a similar searching analysis is applied to any other data that result from interferometric measurements made at the median point when three individual tripod spacers are used.

In the lower section of figure 3 are plotted the expansivities of the 10 different samples that were reported on by Austin and Pierce [8]. These expansivities are represented by open circles, and the small numbers directly to the right and left of the circles represent the numbers assigned by the authors to the corresponding samples. According to them, "these data were derived from a curve representing a graphical average of the observations which include at least 30 points for each sample." The relative magnitudes of the expansivities represented by these points change considerably with temperature, and it is reasonable to assume that the differences in physical conditions

of the samples may have been partly responsible for these changes, but it is improbable that they were the only cause. It is believed that the total variation in impurity of these samples did not exceed the variation in total impurity of samples A, B, and C, which show no no such relative change. Particular attention is called to the results on sample 8, for which the expansivity obtained is highest at 100°, lowest at 200° and 300°, and again highest at 400° C. Values derived from the expansion data of samples A, B, and C (see table 2) are also plotted here for comparison.

TABLE 3.—*True coefficient of linear thermal expansion of high-purity iron with comparative results on less pure irons*

Temperature	Sample A (total impurity 0.008%)	Sample B (total impurity 0.024%)	Sample C (total impurity 0.3%)	Difference (total spread)
°C	×10⁻⁶/°C	×10⁻⁶/°C	×10⁻⁶/°C	×10⁻⁶/°C
0	11.50	11.46	11.59	0.13
50	12.23	12.23	12.31	.08
100	12.87	12.89	12.93	.06
150	13.44	13.49	13.50	.06
200	13.96	14.04	14.01	.08
250	14.45	14.54	14.49	.09
300	14.91	15.02	14.94	.11
350	15.34	15.46	15.37	.12
400	15.75	15.89	15.77	.14

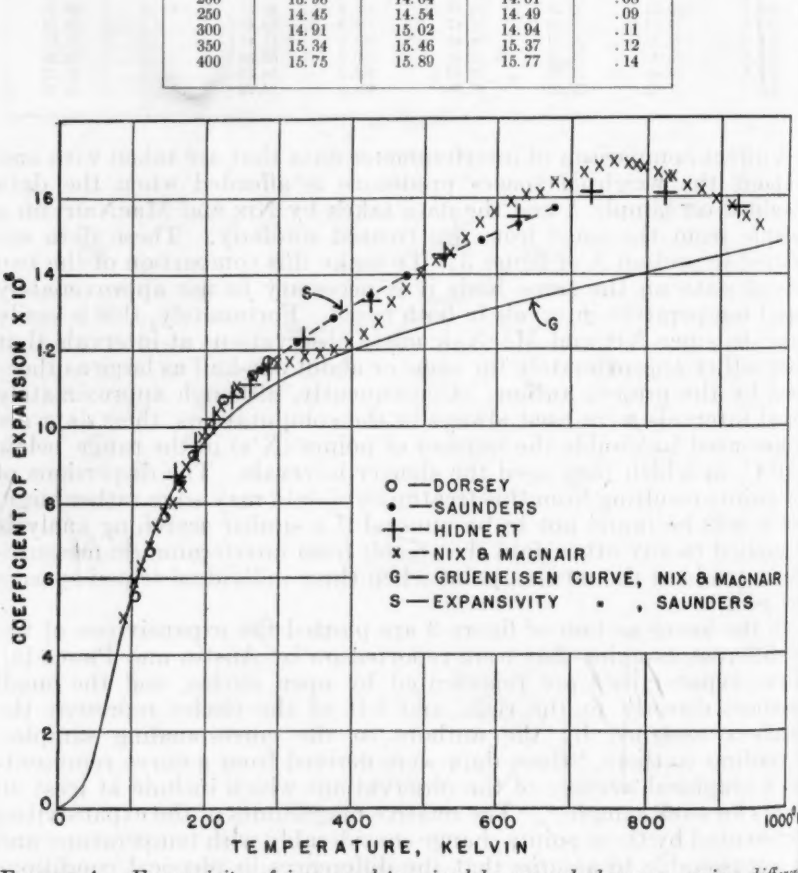


FIGURE 4.—*Expansivity of iron as determined by several observers, using different arrangements of the interferometer, compared with the precision comprator method.*

Figure 4 is a reproduction of Nix and MacNair's figure V [4], on which the results of the present investigation and those of Hidnert and Dorsey have been superimposed. The purpose is to show that the results obtained in the present investigation for the range from room temperature to 400° C are a smooth continuation of the trend followed by the results obtained by others for relatively pure iron in the range below 0° C. This plot also shows that the improved data now obtained are in reasonable agreement with those that Hidnert obtained by a different method [11]. Moreover, it appears that other values than those used by Nix and MacNair for the required constants must be used, if a Grueneisen curve is obtained that will conform with the expansivity as temperature changes.

VI. CONCLUSIONS

1. According to the results obtained, impurities do not affect materially the expansivity of iron in the range from room temperature to 400° C as long as the carbon content is less than 0.05 percent and the total of the usual impurities does not exceed 0.3 percent.

2. In these precise measurements there are no appreciable irregularities in the temperature range from room temperature to 400° C.

3. The precision of the interferometer method has been greatly increased by making observations at a point directly over the contacts between the interferometer plates and a stable spacer.

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A COMPARISON OF PLATINUM AND PALLADIUM HYDROGEN-ELECTRODES IN AQUEOUS SOLUTIONS OF ACID POTASSIUM PHTHALATE

By Walter J. Hamer and S. F. Acree

ABSTRACT

A study was made of the reproducibility of hydrogen electrodes of various types and of silver-silver-chloride electrodes of the thermal-electrolytic type in aqueous solutions of phthalates, in order to learn whether phthalates are reduced to hexahydrophthalic acid and the electromotive force thereby changed. If so, glass electrodes would have to be used to determine the pH values of solutions of phthalates. This type of study is necessary for all reducible materials. Electromotive-force measurements of the electrode combinations were made for various periods of time. The potentials of hydrogen electrodes prepared with platinum sponge under a variety of conditions constantly increased with time and frequently were erratic, whereas those made with palladium sponge under different conditions remained remarkably constant for periods of 35 hours. Even after 75 hours, the potentials increased only 0.3 mv—which corresponds to an increase of only 0.005 in pH. Palladium or platinum electrodes having the same type of coating but of different ages agreed in potential after 2 hours in phthalate solutions. The rate and the magnitude of the increase in potential for the platinum electrodes after approximately 2 hours depend mainly upon the thickness of the metallic sponge. The characteristics of the palladium electrodes were practically independent of the thickness of the metallic sponge; of the current density used in the electrolysis; of the concentration, composition, acidity, and conductance of the plating solution; and of the concentration, composition, pH, and buffer capacity of the phthalate solutions in which the electrodes were used. The difference in the behavior of platinum and palladium hydrogen-electrodes may be caused by their different catalytic activity.

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I. INTRODUCTION

Acid potassium phthalate is commonly used in the preparation of aqueous buffer solutions of known pH values. These are used in the calibration of various types of pH assemblies, especially those composed of glass and calomel electrodes. The pH values of these solutions have been determined from the measurements of the emf

of galvanic cells comprised of the solution and hydrogen and calomel electrodes. The calomel electrode entails the use of a liquid junction whose potential cannot be determined on a thermodynamic basis. The present pH scale is based upon emf measurements of galvanic cells of this type for a series of pure and stable buffer solutions.[1] For the measurements to have significance, the cells must necessarily be reproducible, the emf must be independent of time, the electrodes must exhibit no temperature or pressure hysteresis, and the buffers must undergo no chemical reactions of any type, such as oxidation or reduction. For measurements with acid potassium phthalate there is considerable doubt as to whether these conditions can be attained.

Merrill [1] [2], Oakes and Salisbury [2], Draves and Tartar [3], Kolthoff and Tekelenburg [4], Hitchcock and Taylor [5], and most recently MacInnes, Belcher, and Shedlovsky [6] stated that the emf obtained with solutions of acid potassium phthalate are not independent of time but show a gradual increase. This change, if not preventable, would exclude acid potassium phthalate as a possible pH standard. Draves and Tartar found that the hydrogen electrode completely reduced within 122 hours a 0.2–*M* solution of acid potassium phthalate to the salt of hexahydrophthalic acid.[3] The emf rose during reduction because the ionization constant of hexahydrophthalic acid is lower than that for *o*-phthalic acid. These authors suggested that the potassium hexahydrophthalate would make a better pH standard than the acid potassium phthalate because of its stability (completely reduced state). MacInnes, Belcher, and Shedlovsky found that the increase in the emf was more pronounced at 40° than at 25° C and atttrbuted this to an increase in the rate of reduction of the phthalate. Because of the uncertainty in their measurements with hydrogen electrodes, they obtained the pH value of a 0.05–*M* solution of acid potassium phthalate by means of a calibrated glass electrode. On the other hand, Acree and Knight [7] with acid sodium phthalate, and Clark and Lubs [8], Wood and Murdick [9], Russell and Stauffer [10], and Bacon, Hensley, and Vaughn [11] with acid potassium phthalate found that the emf was practically independent of time. Causes for these differences in observations still remain unexplained.

Draves and Tartar stated that the extent of the drifts in the emf depends on the thickness of the metallic sponge used in the preparation of the hydrogen electrodes. Oakes and Salisbury, and Hitchcock and Taylor believed that certain impurities catalyzed the reduction of the phthalate at the surface of the hydrogen electrodes. Oakes and Salisbury found that drifting potentials were obtained even after repeated recrystallizations of the buffer from water, whereas Hitchcock and Taylor reported satisfactory results after three recrystallizations. Clark [12] believed that the extent of the

[1] Work is now in progress at this Bureau on a pH scale based upon emf measurements of galvanic cells without liquid junctions. See W. J. Hamer and S. F. Acree, J. Research NBS **23**, 647 (1939) RP1261, and W. J. Hamer, J. O. Burton, and S. F. Acree, J. Research NBS **24**, 269 (1940) RP1284 for descriptions of the method, and R. G. Bates, W. J. Hamer, G. G. Manov, and S. F. Acree, J. Research NBS **29**, 183 (1942) RP1495 for buffer solutions fixing the pH scale from approximately 2 to 12.

[2] Figures in brackets indicate the literature references at the end of this paper.

[3] No attempts were made in this investigation to isolate the hexahydrophthalic acid in view of the fact that Draves and Tartar recovered this acid from the solutions by acidification with hydrochloric acid. They found that the dry acid and its anhydride melted at 192° and 31.5° C, respectively, in agreement with the melting points for these compounds found by Baeyer, Liebigs Ann. Chem. **258**, 219 (1890). Also Willstätter and Bequet, Ber. deut. chem. Ges. **51**, 767 (1918), reduced free phthalic acid in glacial acetic acid by hydrogen gas in the presence of platinum black.

drifts in the emf may have been due to the type of material used in the construction of the hydrogen electrodes. To investigate this possibility he compared an iridium electrode contained in one vessel with a platinum and a palladium electrode in another vessel. He found that these functioned similarly and that the potentials increased about 1 mv in 24 hours. However, since the platinum and palladium electrodes were used in the same vessel, no independent comparison of them was obtained. If the platinum electrode had catalyzed the reduction of acid potassium phthalate, thereby causing a change in the hydrogen-ion concentration of the solution, the potential would increase in accordance with the Nernst equation to correspond with the change in hydrgoen-ion concentration. The statement by Beans and Hammett [13], that if two or more hydrogen electrodes in the same solution agree in potential, their common potential is a measure of the true hydrogen-ion concentration, is true only if the electrodes have not been exposed to air and exhibit no drifts in potential with time. Acree and associates have shown [14] that electrodes prepared of platinum, palladium, iridium, and tin behave quite differently, especially in solutions which may be easily reduced at the surface of hydrogen electrodes, such as dimethylaniline hydrochloride and hydroxylamine. Slagle and Acree stated that palladium is to be preferred for use in solutions which may undergo reductions. Until now, however, no exact comparison of the various types of hydrogen electrodes has been made.

Certain experimenters have attributed the drifts in the emf to a constantly changing potential at the junction between the solutions of acid potassium phthalate and the potassium chloride of the calomel half-cell. However, it has been shown for other materials that no drifts occur when properly constructed liquid junctions are used [15 to 21]. Russell and Stauffer have recently shown that this is also true for solutions of acid potassium phthalate. The design of the cell is therefore a factor that must be considered. Another possibility that may account for the lack of constancy of the emf [15] is the age of the hydrogen and calomel electrodes. It has been shown [18, 19, 20, 21] that if these electrodes are not sufficiently aged, they will give different potentials in solutions of the same composition. Smith and Taylor [22] have likewise shown that if silver–silver-chloride electrodes are not sufficiently aged, new electrodes will act as cathodes toward aged ones when immersed in a chloride solution and that periods as long as 1 week may be required for attainment of a common potential. Consequently, drifts in the emf will occur for this duration unless some means is found to eliminate the cause.

In view of these conflicting opinions and data, solutions of acid potassium phthalate cannot be certified as pH standards nor can a thermodynamic study be made of mixtures of *o*-phthalic acid and potassium hydroxide until it is established that precise measurements can be made with hydrogen electrodes. This paper presents the results of a systematic study of the behavior of hydrogen electrodes in acid potassium phthalate in which the various factors mentioned above have been investigated. Measurements were made at temperatures other than 25° C in order to ascertain the effects of such changes. Measurements were also made with acid potassium phthalate mixed with *o*-phthalic acid and with potassium hydroxide in order

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to determine whether the drifts in the emf observed with acid potassium phthalate could be caused by its relatively low buffer capacity [13]. Measurements were made by means of cells without liquid junction and with pure materials, thus eliminating two of the causes of the uncertainties mentioned above.

II. EXPERIMENTAL PROCEDURES

1. MATERIALS AND SOLUTIONS

All the tests were made with acid potassium phthalate, National Bureau of Standards Standard Sample 84a, of a certified purity of 100.00 percent determined by titration. It was prepared for use according to the directions given in the Bureau certificate.

Solutions containing various amounts of acid potassium phthalate and *o*-phthalic acid were prepared from known weights of the dry salt, dry acid, and conductivity water. The *o*-phthalic acid was prepared from resublimed phthalic anhydride that gave less than 0.002 percent of ash when ignited. The phthalic anhydride was boiled with distilled water after the manner of Van de Stadt [23]. The acid was then recrystallized three times from distilled water and extracted with benzene in a Soxhlet extractor. After removal of the benzene, the acid was boiled with distilled water to remove any traces of anhydride that may have been formed and was finally recrystallized from water. After air-drying, the crystals were lightly ground in an agate mortar and dried in vacuum at room temperature over anhydrous magnesium perchlorate. Two lots of the acid were prepared, and the purity of each was checked by weight titration in an atmosphere free of carbon dioxide, using CO_2-free sodium hydroxide, standardized against acid potassium phthalate, National Bureau of Standards Standard Sample 84a. Phenolphthalein solution was used as the indicator. The purity of one lot of the acid as indicated by the titrations was 100 percent, and that of the other was 99.85 percent. However, when samples of the second lot were carefully ground and redried the calculated purity rose to 100 percent. Hence it was assumed that the impurity was water entrapped in the crystals, and allowance was made for it in the preparation of the solutions.

Solutions containing acid potassium phthalate and dipotassium phthalate were prepared from the requisite amounts of dry acid potassium phthalate and standardized potassium hydroxide, since dipotassium phthalate is extremely hygroscopic. The standardized solution of potassium hydroxide was prepared by dilution with known weights of conductivity water of a 50-percent solution of potassium hydroxide from which the carbonate was removed by the dropwise addition of a saturated solution of barium hydroxide until no more precipitate of barium carbonate was formed. It was standardized against benzoic acid, National Bureau of Standards Standard Sample 39e, using phenolphthalein solution as the indicator. Results of 5 titrations agreed within 0.02 percent.

Potassium chloride was recrystallized twice from conductivity water and then thrice recrystallized from 95-percent ethanol by the method of Hahn [24], to remove the last traces of bromide. It was dried for at least 10 hours at 110° C and allowed to cool in a desiccator, using concentrated sulfuric acid as the desiccant.

After the solutions were prepared, they were deaerated by means of hydrogen gas, corrections being made for loss of water from the solutions and the differences between the densities of hydrogen and air. The specific conductance of the water used for these studies was 1.0×10^{-6} mho, and sometimes was as low as 0.6×10^{-6} mho.

2. PREPARATION OF THE ELECTRODES

Silver–silver-chloride electrodes of the thermal-electrolytic type were prepared from pure materials, as described by Hamer and Acree [25]. They were aged in distilled water in an attempt to bring them to a stable state. Some were stored in distilled water for 10 to 12 hours, some for 1 month, and a few for 3 months. All were preliminarily tested in 0.05–*N* hydrochloric acid and they usually agreed within 0.10 mv.

In order to test the effects of the nature and thickness of the metallic sponge on the performance of hydrogen electrodes, several types were studied. They may be grouped into two main categories—those made of platinum and those made of palladium. The platinum electrodes were constructed of platinum foil, 10 by 15 by 0.2 mm, welded to a platinum wire sealed in the end of a glass tube. The foil was cleaned for about 3 minutes in aqua regia diluted with an equal volume of distilled water, and then washed thoroughly in distilled water. The foil was then plated with platinum black in a chloroplatinic acid solution, designated as solution Pt_A, composed of 3 g of platinum in 100 ml of 0.25–*N* hydrochloric acid and 1 ml of a 5-percent solution of lead acetate. In every case the addition of lead acetate was found necessary. This solution had a specific conductance of 0.163 mho and a pH of 0.84 at 30° C. A current of 250 ma or 400 ma was used for a definite period of time. Electrodes designated $Pt_{1A(.08)}$, $Pt_{3A(.08)}$, and $Pt_{5A(.08)}$ were plated, respectively, for 1.0, 3.0, and 5.0 minutes at 250 ma or at a current density of 0.08 amp/cm². Here and elsewhere in the text, in the subscript describing the hydrogen electrode the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parenthesis refer to the current density at which the electrode was plated. Those designated $Pt_{1A(.13)}$ and $Pt_{5A(.13)}$ were plated for 1.0 and 5.0 minutes at 400 ma, or at a current density of 0.13 amp/cm². Making a conservative estimate of a current efficiency of 50 percent, electrodes $Pt_{1A(.08)}$, $Pt_{3A(.08)}$, $Pt_{5A(.08)}$, $Pt_{1A(.13)}$, and $Pt_{5A(.13)}$ were covered, respectively, with 0.016, 0.046, 0.076, 0.021, and 0.122 g of platinum. Assuming uniform distribution of platinum on the surface of the foil, the thickness of the sponge (calculated as solid metal) was approximately 2, 6.9, 11, 3.2, and 17 microns, respectively.[4] In all cases, the sponge was finely divided and was jet black in appearance.

A few platinum electrodes, designated as $Pt_{1B(.08)}$, $Pt_{3B(.08)}$, $Pt_{5B(.08)}$, $Pt_{1B(.13)}$, and $Pt_{5B(.13)}$, were prepared from 50 ml of the solution Pt_A of chloroplatinic acid, diluted with 50 ml of conductivity water. This solution, designated Pt_B, had a conductivity of 0.089 mho and a pH of 1.18 at 30° C, and it was therefore less acidic and more resistant to current flow than the former. Solution Pt_B also yielded finely divided sponge, jet black in appearance.

[4] Studies of the capacitance and surface characteristics of the metals of the platinum group are being made that will give more information of the effective thickness and surface area of these electrodes.

The palladium electrodes were constructed of platinum foil, 10 by 15 by 0.2 mm, welded to a platinum wire sealed in the end of a glass tube. They were cleaned as above or in dilute hydrochloric acid and thoroughly washed in distilled water. The foil was then plated with palladium black in four different solutions, described below, by passing a current of 250 or 400 ma for a definite period of time. Electrodes designated as $Pd_{1(.08)}$, $Pd_{3(.08)}$, and $Pd_{5(.08)}$ were plated, respectively, for 1.0, 3.0, and 5.0 minutes at 250 ma, or at a current density of 0.08 amp/cm². Electrodes designated $Pd_{1(.13)}$ and $Pd_{5(.13)}$ were plated for 1.0 and 5.0 minutes at 400 ma or at a current density of 0.13 amp/cm². Making a conservative estimate of a current efficiency of 50-percent, electrodes $Pd_{1(.08)}$, $Pd_{3(.08)}$, $Pd_{5(.08)}$, $Pd_{1(.13)}$, and $Pd_{5(.13)}$, were covered, respectively, with 0.008, 0.025, 0.042, 0.011, and 0.067 g of palladium. Assuming uniform distribution of palladium on the surface of the foil, the thickness of the coats (calculated as solid metal) of these five electrodes was approximately 2.4, 7.1, 11.8, 3.8, and 18.9 microns, respectively, or approximately the same thickness as the coats on the platinum electrodes. A few electrodes prepared of palladium foil, 5 by 10 by 0.2 mm, were also tested. These electrodes, designated respectively by the symbols $Pd'_{1B(.25)}$ and $Pd'_{5B(.25)}$ were plated with palladium black by electrolysis in palladium solution Pd_B (see below), using 250 ma for 1 and 5 minutes.

One solution used for the electrolysis was prepared by the dilution of 50 ml of H_2PdCl_4 solution with 40 ml of distilled water and 5 ml of 1.0–*N* hydrochloric acid. This solution, designated Pd_A, had a palladium content of 5.05 percent, a pH of 1.31, and a specific conductance of 0.4 mho. Electrodes prepared with its use were designated by the subscript A. A second solution was prepared by the dilution of 38 ml of Pd_A solution with 152 ml of distilled water and 10 ml of 1.0–*N* hydrochloric acid, to which 0.053 g of lead acetate was added. This solution, designated as Pd_B solution, contained 1.04 percent of palladium, had a specific conductance of 0.384 mho and a pH of 1.23. A solution of Na_2PdCl_4 containing 1 g of palladium per liter was tried. No palladium black could be obtained from this solution, even upon the addition of 1 ml of 5-percent lead acetate solution. However, upon the addition of 10 ml of 1.0–*N* hydrochloric acid to 100 ml of this solution, a finely divided deposit of palladium black was readily obtained by electrolysis. This solution, designated as Pd_C solution, contained 0.09 percent of palladium, had a pH of 1.04, and a specific conductance of 0.3 mho. A fourth solution was prepared from 30 ml of Pd_C solution by the addition of 10 ml of concentrated hydrochloric acid. This solution, designated as Pd_D solution, contained 0.068 percent of palladium, had a pH of 0.52, and a specific conductance of 0.155 mho. Solutions Pd_A and Pd_C gave fine deposits that were grayish brown in appearance, whereas solution Pd_D gave a very coarse or granular deposit that was likewise grayish brown. Solution Pd_B gave a very fine and smooth black deposit.

All the hydrogen electrodes were thoroughly rinsed in distilled water immediately after they were plated with sponge. Some were used immediately. Others were stored in distilled water for several days to several months. A few were stored in distilled water for a year before being used. Those in each age group were always checked

against each other in 0.05–*N* hydrochloric acid before use. In all cases, the electrodes agreed within 0.1 mv after 1 hour and within 0.03 mv after 4 hours.

3. APPARATUS

The details of the apparatus used in these studies, including the cells, bubble tubes, electrodes, thermostat, and the emf recording instruments have been described by Hamer and Acree [25]. The cells consisted of two compartments, one for the hydrogen electrodes and one for the silver–silver-chloride electrodes. The volumes of the compartments for the hydrogen and silver–silver-chloride electrodes were 35 and 100 ml, respectively. The cells and bubble tubes were filled under reduced pressure, the temperature of the thermostat was controlled within 0.01° C at all the temperatures, and the emf was measured by means of a calibrated potentiometer with galvanometer and standard cell. The electromotive forces were corrected in the usual manner [26] to 1 atmosphere of hydrogen pressure. The hydrogen gas was purified for these studies by the method described by Hamer and Acree [25].

III. BEHAVIOR OF HYDROGEN ELECTRODES IN AQUEOUS SOLUTIONS OF PHTHALATES

All tests were made with galvanic cells without liquid junction, using hydrogen and silver–silver-chloride electrodes. Hydrogen electrodes consisting of platinum foil covered with platinum sponge were first studied at 25° C in solutions containing 0.05–*m* acid potassium phthalate and 0.05–*m* potassium chloride. The addition of the chloride is a prerequisite for the use of the silver–silver-chloride electrodes in such solutions.

The emf were measured at definite intervals of time after the flow of the hydrogen gas was started, and are recorded in table .1 The emf increased rapidly during the first hour, which is the time required for attainment of equilibrium. However, in all cases the emf continually increased, the rate of the increase being somewhat more marked for electrodes prepared with thicker coats of platinum black. The current density used in the formation of the platinum black seems not to be critical however, since electrodes $Pt_{1A(.08)}$ and $Pt_{1A(.13)}$ and electrodes $Pt_{5A(.08)}$ and $Pt_{5A(.13)}$ had coats of about the same thickness but were plated at different current densities, 0.08 amp/cm² for the former and 0.13 amp/cm² for the latter electrodes.

After 5 to 6 hours, electrodes prepared with the thicker coats behaved irregularly. This performance may be the result of a diffusion layer at the electrode surface. If the acid potassium phthalate in the vicinity of the electrode is rapidly reduced and is continually mixing with unreduced material, the solution near the electrode continually changes in composition, giving rise to erratic behavior. The "rocking type" of hydrogen electrode used by Clark [27] would eliminate this behavior, and the use of this electrode undoubtedly accounts for the fact that he did not observe fluctuations in the emf.

The electrodes were removed and replaced with thinly plated ones. Although the latter performed better, no constant value of the emf could be obtained with their use. However, the drifts were not very marked, amounting to a change of only 0.03 in pH in 24 hours for

TABLE 1.—*Results obtained with hydrogen electrodes made with platinum sponge*

Hydrogen electrodes were aged ½ hour; silver–silver-chloride electrodes were aged 12 hours. Electromotive forces of the galvanic cell Pt, H_2(g)/KHPh (0.05 *M*), KCl (0.05 *M*)/AgCl(s)/Ag(s) at 25° C for definite intervals of time after the flow of hydrogen gas was started.]

Time	$Pt_{1A(-.08)}$ [a]	Time	$Pt_{2A(-.08)}$	Time	$Pt_{3A(-.08)}$	Time	$Pt_{1A(-.13)}$	Time	$Pt_{3A(-.13)}$	Time	$Pt_{1B(-.08)}$	Time	$Pt_{3B(-.13)}$
hr	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*
0.25	0.53871	0.25	0.53831	0.25	0.53852	0.25	0.53817	0.25	0.53823	0.25	0.53867	0.25	0.53869
.50	.53938	.50	.53927	.50	.53947	.50	.53901	.50	.53899	.50	.53931	.50	.53922
.75	.53981	.75	.53992	.75	.53975	.75	.53990	.75	.53973	.75	.53985	.75	.53985
1.0	.53992	1.0	.53993	1.0	.53998	1.0	.53988	1.0	.53998	1.0	.53999	1.0	.54002
2.5	.53995	2.5	.54010	2.5	.53999	2.5	.53991	2.5	.54003	2.5	.53999	2.5	.54033
3.5	.53999	3.5	.54072	3.5	.54006	3.5	.53995	3.5	.54124	3.5	.54000	3.5	.54061
5.0	.54001	5.0	(b)	5.0	.54021	5.0	.53999	5.0	.54316	5.0	.54006	5.0	.54067
6.0	.54007			6.0	.54044	6.0	.54004	6.0	(b)	6.0	.54009	6.0	.54075
7.0	.54013		$Pt_{1A(-.08)}$	7.0	(b)	7.0	.54009			7.0	.54018	7.0	.54079
9.0	.54023					9.0	.54019		$Pt_{1A(-.13)}$	9.0	.54021	9.0	.54092
10.0	.54035				$Pt_{1A(-.08)}$	10.0	.54023			10.0	.54022	10.0	.54093
11.0	.54047					11.0	.54029			11.0	.54027	11.0	.54101
12.0	.54061	1.0	0.54002			12.0	.54044			12.0	.54041	12.0	.54127
24.0	.54187	2.0	.54077			24.0	.54133	0.5	0.54004	24.0	.54153	24.0	.54265
		3.0	.54082	0.50	0.53991			1.5	.54019				
		4.0	.54082	1.5	.54042			2.5	.54021				
		5.0	.54091	2.5	.54051			3.5	.54021				
		6.0	.54099	3.5	.54057			4.5	.54033				
				4.5	.54063			5.5	.54037				
				12.5	.54109			13.5	.54113				

[a] Cells drained of solution and refilled. Hydrogen electrodes aged 24 hours; silver–silver-chloride electrodes 36 hours

Cells drained of solution and refilled. Hydrogen electrodes aged 24 hours; silver–silver–chloride electrodes 36 hours

Time	$Pt_{1A(-.08)}$	Time	$Pt_{3A(-.08)}$	Time	$Pt_{5A(-.08}$	Time	$Pt_{1A(-.13)}$	Time	$Pt_{5A(-.13)}$	Time	$Pt_{1B(-.08)}$	Time	$Pt_{5B(-.13)}$
hr	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*	*hr*	*v*
0.25	0.53844	0.25	0.53871	0.25	0.53889	0.25	0.53905	0.25	0.53897	0.25	0.53888	0.25	0.53897
.50	.53907	.50	.53966	.50	.53926	.50	.53971	.50	.53977	.50	.53981	.50	.53986
.75	.53986	.75	.53990	.75	.53981	.75	.53992	.75	.53994	.75	.53997	.75	.54000
1.0	.53979	1.0	.53993	1.0	.53988	1.0	.53992	1.0	.53999	1.0	.53997	1.0	.54002
2.0	.53988	2.0	.53995	2.0	.54002	2.0	.54001	2.0	.54007	2.0	.53998	2.0	.54011
3.0	.53999	3.0	.54042	3.0	.54010	3.0	.54007	3.0	.54023	3.0	.53999	3.0	.54029
4.0	.54001	4.0	.54056	4.0	.54051	4.0	.54007	4.0	.54016	4.0	.53999	4.0	.54044
5.0	.54001	5.0	.54059	5.0	.54029	5.0	.54006	5.0	.54091	5.0	.54003	5.0	.54057
6.0	.54004	6.0	.54064		(b)	6.0	.54014	6.0	(b)	6.0	.54007	6.0	.54061
7.0	.54011	7.0	.54069			7.0	.54018			7.0	.54012	7.0	.54077
8.0	.54013	8.0	.54077			8.0	.54021			8.0	.54016	8.0	.54090
9.0	.54017	9.0	.54083		$Pt_{5B(-.08)}$	9.0	.54022		$Pt_{5B(-.13)}$	9.0	.54023	9.0	.54108
10.0	.54018	10.0	.54091			10.0	.54022			10.0	.54030	10.0	.54123
20.0	.54044	20.0	.54220			20.0	.54067			20.0	.54071	20.0	.54198
				0.5	0.53886			0.5	0.53957				
				1.5	.53971			1.5	.53999				
				2.5	.53997			2.5	.54029				
				13.5	(c)			2.5	.54029				
								13.5	(c)				

[a] In the subscript describing the hydrogen electrode, the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parentheses refer to the current density at which the electrode was plated. For example, the electrode $Pt_{1A(-.08)}$ was made of platinum sponge and was plated for 1 minute, using solution Pt_A at a current density of 0.08 amp/cm.[2]

[b] Cell became erratic and hydrogen electrode was replaced by one of a different type.

[c] Cell became erratic; not replaced by a new electrode.

the thinly coated electrodes and to only 0.01 in pH after 3.5 hours for the heavier coated ones. The rate of increase was somewhat less than that found by Draves and Tartar. However, they employed only 15 ml of solution whereas in this work 35 ml was used, and 100 ml more was available in the silver–silver-chloride compartment for diffusion of the phthalate to the hydrogen electrode. This fact may account in part for the differences in data reported in the literature. The extent of the drifts in the emf depends upon the amount of solution used in the hydrogen electrode compartment and is less marked for large volumes of solution. Hence, design of the cell is one of the factors that must be considered in the use of hydrogen electrodes in phthalate solutions.

In view of the small drifts in the emf, it was considered possible that the electrodes may not have been in a stable state. Consequently, the cells were drained of solution and refilled. Hydrogen electrodes aged for 1 day and silver–silver-chloride electrodes aged for 36 hours were then used. Similar results were observed with the aged electrodes. The emf obtained with them also agreed almost perfectly with the emf obtained with new ones. Evidently, the small drifts in the observed emf cannot be explained by aging effects. Smith and Taylor have shown that the aging effects for silver–silver-chloride electrodes arise from concentration polarization. The process of filling the cells under reduced pressure used in these studies undoubtedly reduces substantially the differences in concentration of solution inside and outside the pores of the silver–silver-chloride electrodes, thus decreasing the cause of the aging effects. Data of table 3 for silver–silver-chloride electrodes aged 12 hours or a week likewise show that the age of the silver–silver-chloride electrodes is not responsible for the drifts in the emf.

The possibility that the composition of the solution of chloroplatinic acid used in the preparation of the platinum sponge may be responsible for electrode characteristics was next briefly investigated. Electrodes were plated with platinum sponge, using solution Pt_B. The data are recorded in the last two columns of table 1. The emf drifts and other characteristics were the same for these electrodes as were observed for those plated in solution Pt_A.

Hydrogen electrodes of platinum foil covered with palladium sponge were next studied under identical conditions. The results are given in table 2. The emf remained remarkably constant for all thicknesses of palladium sponge. Only after 34 hours were drifts observed, and even after 75 hours of continual use the increase in the emf corresponded to a change in pH of only 0.005. This is true of fine (solution A) and coarse (solution D) deposits and for sponges deposited at different current densities. For example, electrodes $Pt_{1A(.08)}$ and $Pd_{1D(.08)}$ were plated at a current density of 0.08 amp/cm^2, and $Pd_{5A(.13)}$ and $Pd_{5D(.13)}$ at a current density of 0.13 amp/cm^2. No significant differences were noted for electrodes prepared with platinum and palladium foil (compare columns 12 and 14 with other columns of table 2) or plated in solutions of different composition. The electrode characteristics are therefore determined primarily by the metal composing the spongy coating on the electrode.

From these few measurements it would appear that palladium sponge is preferable to platinum sponge for use in solutions of acid potassium phthalate at 25° C. This is probably due to the lower catalytic

TABLE 2.—*Results obtained with hydrogen electrodes made with palladium sponge*

[Hydrogen electrodes were aged ½ hour; silver–silver-chloride electrodes were aged 12 hours. Electromotive forces of the galvanic cell Pd, H_2 (g)/KHPh (0.05 *M*), KCl (0.05 *M*)/AgCl (s)/Ag (s) at 25° C for definite intervals of time after the flow of hydrogen gas was started]

Time	[a]$Pd_{1A(.05)}$	Time	$Pd_{2A(.05)}$	Time	$Pd_{3A(.05)}$	Time	$Pd_{1A(.15)}$	Time	$Pd_{3A(.15)}$	Time	[b]$Pd'_{1B(.45)}$	Time	$Pd'_{1B(.45)}$
hr		*hr*		*hr*		*hr*		*hr*		*hr*		*hr*	
0.25	0.53861	0.25	0.53887	0.25	0.53862	0.25	0.53827	0.25	0.53892	0.25	0.53873	0.25	0.53900
.50	.53932	.50	.53951	.50	.53943	.50	.53909	.50	.53957	.50	.53949	.50	.53973
.75	.53968	.75	.53983	.75	.53980	.75	.53981	.75	.53990	.75	.53983	.75	.53994
1.0	.53979	1.0	.53991	1.0	.53984	1.0	.54000	1.0	.53993	1.0	.53997	1.0	.53995
2.5	.53992	2.5	.53993	2.5	.53996	2.5	.54002	2.5	.54000	2.5	.53997	2.5	.53996
3.5	.53993	3.5	.53995	3.5	.53997	3.5	.54000	3.5	.53999	3.5	.53998	3.5	.53996
5.0	.53990	5.0	.53997	5.0	.53997	5.0	.54000	5.0	.53998	5.0	.53999	5.0	.53996
6.0	.53991	6.0	.53996	6.0	.53997	6.0	.54004	6.0	.54000	6.0	.53997	6.0	.53997
7.0	.53992	7.0	.53997	7.0	.53999	7.0	.54002	7.0	.53998	7.0	.53997	7.0	.53996
9.0	.53995	9.0	.53999	9.0	.54001	9.0	.54001	9.0	.53998	9.0	.53999	9.0	.53997
10.0	.53994	10.0	.54000	10.0	.54000	10.0	.54000	10.0	.53996	10.0	.53999	10.0	.53997
	(c)	12.0	.54001		(c)	(c)		12.0	.53999		(c)		(c)
	$Pd_{1D(.05)}$	22.0	.54007		$Pd_{3D(.05)}$		$Pd_{3D(.15)}$	22.0	.54001		$Pd_{1B(.05)}$		$Pd_{1B(.05)}$
0.5	0.53927	34.0	.54001	0.5	0.53594	0.5	0.53807	34.0	.54004	0.5	0.53968	0.5	0.53980
1.5	.53990	51.0	.54007	1.5	.53998	1.5	.53996	51.0	.54017	1.5	.54003	1.5	.54007
12.5	.53988	55.5	.54016	12.5	.54001	12.5	.53999	55.5	.54023	12.5	.54000	12.5	.54008
24.5	.53997	75.0	.54022	24.5	.53996	24.5	.53994	75.0	.54032	24.5	.54006	24.5	.54011
41.0	.54003			41.0	.54007	41.0	.54000			41.0	.54009	41.0	.54017

[a] In the subscript describing the hydrogen electrode, the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parentheses refer to the current density at which the electrodes were plated. For example, the electrode $Pd_{1A(.05)}$ was made of palladium sponge and was plated for 1 minute, using solution Pd A at a current density of 0.05 amp./cm².

[b] Electrodes designated with a prime (columns 12 and 14) were made with palladium foil, whereas the others not primed were made of platinum foil.

[c] Hydrogen electrode was replaced by one of a different type.

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activity of palladium. In studies of catalytic reduction, Lorch [28] found that palladium has a much lower activity than platinum or iridium. Although it is well known that palladium adsorbs more hydrogen than platinum of iridium, it is not the amount of hydrogen that is adsorbed but the catalytic activity that determines the effectiveness of a hydrogen electrode. Popoff, Kunz, and Snow [29] found that electrodes given a preliminary plating of gold, as recommended by Lewis, Brighton, and Sebastian [30] because gold is impervious to hydrogen and therefore should come to equilibrium more quickly, showed no differences from electrodes not plated with gold.

In order to determine whether the same characteristics prevail at other temperatures, similar measurements were made at 0°, 45°, and 60° C, using a new pair of hydrogen and silver–silver-chloride electrodes and a pair aged for 1 week. The data recorded in table 3 show that similar conclusions regarding the age of the electrodes and the relative behavior of platinum and palladium apply to these temperatures as well. A slight increase in the emf was noted at 60° C after 4 hours, which may indicate reduction of the phthalate. However, other results given in later tables show less drifting at 60° C. Two series of measurements were made at 0° C. In series A, the flow of hydrogen gas was started within 3 minutes after the temperature of the bath reached 0° C. In series B, the flow of hydrogen gas was started 3 hours later. For series A, the emf increased steadily during the first 4 hours, whereas for series B the equilibrium values were obtained within ½ to 1 hour. In series A, the initial increase in the emf was not due to reduction of the phthalate but to the time required to attain temperature equilibrium within the cell compartments. It was also observed in this series of experiments that electrodes which at first behaved erratically functioned properly when retained long enough in the solutions.

Most of the observations reported in the literature on the behavior of hydrogen electrodes are for aqueous solutions of phthalates alone, whereas the above observations were for 0.05–*m* acid potassium phthalate containing 0.05–*m* potassium chloride. In order to determine whether the presence of potassium chloride had any influence upon electrode behavior, a series of measurements were made at 25° C for solutions containing various amounts of potassium chloride. The results are given in table 4. Three equilibrium values obtained at 25° C with palladium electrodes are given as *a*, *b*, *c*. The first set of values was obtained after 5 hours of hydrogen flow, the second was taken 44 hours later, and the third 60 hours later. The first two sets agree within 0.05 mv, and the third set was about 0.1 mv higher than the second. After the third set of readings had been obtained at 25° C, the palladium electrodes were replaced by $Pt_{5A(.08)}$ electrodes aged 1 month. With their use, the emf continually increased, again showing that platinum sponge catalyzes the reduction of acid potassium phthalate, the extent of which is not critically dependent upon the concentration of the potassium chloride. Hence, potassium chloride does not influence the performance characteristics of hydrogen electrodes in solutions of acid potassium phthalate. After 20 hours these were replaced by $Pd_{5A(.08)}$ electrodes. At equilibrium the emf were slightly higher than the final ones obtained with platinum sponge, probably because of a slight dilution of the solutions

by the insertion of the wet $Pd_{5A(.08)}$ electrodes and to hysteresis in the emf during reduction by the platinum electrodes, but remained at the same value for 24 hours. They were then replaced by $Pt_{1A(.08)}$ electrodes and again the emf continually increased, although not to as large an extent as for the $Pt_{5A(.08)}$ electrodes that were covered with thicker coats of sponge.

In tables 5 and 6, similar data are given for platinum and palladium electrodes in solutions containing dipotassium phthalate, acid potassium phthalate, and potassium chloride of pH 4.92 to 5.33 and in solutions containing *o*-phthalic acid, acid potassium phthalate, and potassium chloride of pH 2.73 to 3.16. Each type of electrode

TABLE 3.—*Electromotive forces of the galvanic cell* Pd *or* Pt, H_2(g)/KHPh(0.05 *M*), KCl (0.05 *M*)/AgCl (s)/Ag(s) *at 0°, 45°, and 60° C for definite intervals of time after the flow of hydrogen gas was started*

Temperature	Series A at 0° C [a]		Series B at 0° C [b]		45° C		60° C	
Cell number	1	2	1	2	1	2	1	2
H_2 electrodes	$Pd_{3A(.08)}$ [c]	$Pd_{5^c(.08)}$	$Pd_{3A(.08)}$	$Pd_{5^c(.08)}$	$Pd_{3A(.08)}$	$Pd_{5^c(.08)}$	$Pd_{3A(.08)}$	$Pd_{5^c(.08)}$
Aged	12 hr	1 week	0.5 hr	1 week	12 hr	1 week	12 hr	1 week
Ag-Ag Cl electrodes, aged	12 hr	1 week	12 hr	1 week	12 hr	1 week	12 hr	1 week
Time	Electromotive force							
hr	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*
0.25			0.52703	0.52706	0.55005	0.55002	0.55761	0.55752
.50	0.52628	0.52637	.52713	.52714	.55008	.55009	.55770	.55772
.75					.55009	.55008	.55773	.55775
1.00	.52660	.52666	.52714	.52718	.55011	.55008	.55773	.55775
1.25					.55010	.55008	.55770	.55771
1.50	.52673	.52677	.52714	.52818	.55008	.55009	.55773	.55774
1.75							.55774	.55777
2.00	.52688	.52695	.52717	.52719	.55012	.55011	.55774	.55779
2.50					.55008	.55009	.55774	.55776
3.00	.52702	.52707	.52717	.52719	.55011	.55011	.55773	.55774
3.50					.55012	.55013	.55779	.55776
4.00	.52714	.52720	.52716	.52719	.55009	.55011	.55781	.55780
5.00	.52711	.52719			.55011	.55012	.55786	.55783
6.00	.52717	.52722	.52718	.52719	.55014	.55013	.55797	.55791
7.00	.52712	.52719	.52719	.52721				
8.00	.52717	.52719						
9.00	.52719	.52721						
10.00	.52725	.52729						
14.00	.52717	.52721						

Palladium-sponge hydrogen electrodes replaced by platinum-sponge hydrogen electrodes aged 1 week

H_2 electrodes	$Pt_{1A(.08)}$	$Pt_{5A(.08)}$	$Pt_{1B(.08)}$	$Pt_{5B(.08)}$	$Pt_{1A(.08)}$	$Pt_{5A(.08)}$	$Pt_{1A(.08)}$	$Pt_{5A(.08)}$
hr								
1.0	0.52725	0.52729	0.52733	0.52729	0.55030	0.55021	(d)	0.558
2.0	.52718	(d)	.52718	.52721	.55021	.55027	(d)	(d)
3.0	.52725	.52743	.52725	.52733	(d)	(d)	0.558	.558
5.0	.52732	.52792	.52731	(d)	.55019	(d)	(d)	(d)
6.0	.52729	.52812	.52733	.52809	.55035	.55100	.55849	.55961
7.0	.52737	.52827	.52733	.52777	.55039	.55209	.55871	.55973
8.0	.52739	.52841	.52741	.52868	.55041	.55224	.55886	
10.0	.52746	.52851	.52739	.52888	.55051	.55248		

[a] Flow of hydrogen gas was started within 3 minutes after the temperature of the bath reached 0° C.
[b] Flow of hydrogen gas was started within 3 hours after the temperature of the bath reached 0° C.
[c] In the subscript describing the hydrogen electrode, the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parentheses refer to the current density at which the electrode was plated. For example, the electrode $Pt_{3A(.08)}$ was made of palladium sponge and was plated for 3 minutes, using solution Pd_A at a current density of 0.08 amp/cm².
[d] Cell behaved erratically.

behaves in these solutions substantially as in solutions containing only acid potassium phthalate and potassium chloride. Their behavior therefore is not dependent upon pH, composition, concentration, or buffer capacity of the solution. Some hydrogen electrodes that had been aged for as long as a year were also used in these solutions. They behave in a manner similar to unaged electrodes. These observations as well as those for the ones aged only a month do not substantiate the statement of Andrews [31], that palladium electrodes deteriorate on standing for long periods in water, and that

TABLE 4.—*Electromotive forces of the galvanic cell* Pd *or* Pt; H_2(g)/KHPh (0.05 *M*), KCl (*m*)/AgCl(s)/Ag(s) *at 25° C for definite intervals of time after the flow of hydrogen gas was started*

[Hydrogen electrodes were aged for 2 hours; silver–silver-chloride electrodes were aged for 12 hours]

Molality of potassium chloride. H_2 electrode	0.05 *m* $Pd_{1A(\cdot 08)}$ [a]	0.03 *m* $Pd_{1A(\cdot 08)}$	0.02 *m* $Pd_{1A(\cdot 08)}$	0.01 *m* $Pd_{1A(\cdot 08)}$	0.007 *m* $Pd_{1A(\cdot 08)}$	0.005 *m* $Pd_{1A(\cdot 08)}$	0.003 *m* $Pd_{1A(\cdot 08)}$	0.002 *m* $Pd_{1A(\cdot 08)}$	0.001 *m* $Pd_{1A(\cdot 08)}$
Time	Electromotive force								
hr	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*
(b)	0.53999	0.55363	0.56440	0.58255	0.59173	0.60040	0.61354	0.62396	0.64196
(c)	.53998	.55364	.56435	.58257	.59174	.60042	.61356	.62393	.64190
(d)	.54002	.55369	.56438	.58270	.59184	.60045	.61368	.62403	.64201
	Palladium electrodes replaced by $Pt_{8A(\cdot 08)}$ electrodes—aged 1 month								
1.0	0.54001	0.55358	0.56427	0.58249	(•)	0.60028	0.61355	0.62392	0.64196
2.0	.54063	.55438	(•)	.58322	0.59240	.60086	.61397	.62459	(•)
3.0	(•)	.55461	.56531	.58363	.59279	.60132	.61470	(•)	.64210
6.0	.54131	.55501	.56620	.58400	.59354	.60225	.61537	(•)	.64265
7.0	.54167	.55513	.56633	.58439	.59372	.60239	.61554	.62591	.64288
19.0	.54283	.55663	.56686	.58522	.59466	.60323	.61633	.62674	.64375
20.0	.54290	.55667	.56706	.58534	.59464	.60333	.61642	.62683	.64373
	Platinum electrodes replaced by $Pd_{5A(\cdot 08)}$ electrodes—aged 1 month								
1.0	0.54233	0.55593	0.56693	0.58526	0.59426	0.60277	0.61520	0.62614	0.64332
2.0	.54279	.55654	.56708	.58537	.59444	.60287	.61625	.62647	.64371
4.0	.54295	.55669	.56704	.58536	.59472	.60345	.61654	.62697	.64375
5.0	.54298	.55663	.56704	.58536	.59472	.60337	.61653	.62695	.64380
6.0	.54297	.55666	.56707	.58536	.59473	.60339	.61650	.62696	.64388
7.0	.54298	.55669	.56709	.58537	.59466	.60341	.61652	.62698	.64384
10.0	.54301	.55671	.56706	.58537	.59468	.60345	.61650	.62700	.64391
23.0	.54292	.55663	.56709	.58530	.59463	.60349	.61666	.62695	.64388
24.0	.54292	.55662	.56709	.58530	.59464	.60351	.61669	.62695	.64388
	Palladium electrodes replaced by $Pt_{1A(\cdot 08)}$ electrodes—aged 1 month								
1.0	0.54221	0.55593	0.56703	0.58530	0.59434	0.60310	0.61620	0.62673	0.64333
2.0	.54295	.55617	.56713	.58537	.59457	.60347	.61645	.62710	.64393
4.0	.54298	.55681	.56713	.58538	.59563	.60355	.61645	.62714	.64380
5.0	.54301	.55680	.56717	.58538	.59464	.60355	.61655	.62716	.64387
6.0	.54299	.55687	.56721	.58541	.59466	.60363	.61653	.62730	.64381
10.0	.54313	.55701	.56718	.58550	.59575	.60371	.61659	.62761	.64494
23.0	.54330	.55760	.56747	.58564	.59477	.60385	.61690	.62794	.64474
24.0	.54330	.55755	.56748	.58565	.59477	.60385	.61689	.62797	.64483

[a] In the subscript describing the hydrogen electrode, the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parentheses refer to the current density at which the electrode was plated. For example, the electrode $Pd_{1A(\cdot 08)}$ was made of palladium sponge and was plated for 1 minute, using solution Pd_A at a current density of 0.08 amp/cm.^{2}

[b] First recorded emf 5 hours after the flow of hydrogen gas was started.

[c] Second recorded emf 44 hours later, after measurements at temperatures from 0° to 20° C.

[d] Third recorded emf 105 hours later, after measurements at temperatures from 0° to 60° C.

[•] Erratic.

ones which had stood for 3 days never attain a state of equilibrium within any reasonable length of time. Although it is well known that surface characteristics of electrodes, such as grain size, may change on standing in air or in various solutions, it does not seem likely that distilled water alone should alter the characteristics of palladium or platinum sponge, and the results obtained in this investigation indicate that it does not.

TABLE 5.—*Electromotive forces of the galvanic cell* Pd *or* Pt, $H_2(g)/KHPh(m_1)$, $K_2Ph(m_2)$, $KCl(m_3)/AgCl(s)/Ag(s)$ *at 25° C for definite intervals of time after the flow of hydrogen gas was started*

[Hydrogen electrodes were $Pd_{1B(.08)}$ [a] aged 3 months; silver-silver-chloride electrodes were aged 2 months]

	Molalities and pH values of the solutions									
$m_1 \times 10^3$	7.6605	16.690	23.505	49.454	69.349	95.161	183.81	263.58	415.27	629.63
$m_2 \times 10^4$	7.7041	16.788	23.639	49.736	69.744	95.703	184.86	265.08	417.64	633.21
$m_3 \times 10^4$	7.6712	16.714	23.538	49.524	69.447	98.295	184.07	263.95	415.86	630.51
pH	5.33	5.29	5.27	5.18	5.15	5.08	5.07	5.04	4.99	4.92
Time	Electromotive forces of the solutions									
hr	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*
(b)	0.72333	0.70180	0.69223	0.67082	0.66100	0.65171	0.63190	0.62093	0.60705	0.57800
(c)	.72336	.70181	.69223	.67084	.66101	.65174	.63187	.62097	.60705	.57807
(d)	.72343	.70185	.69230	.67086	.66107	.65174	.63190	.62099	.60716	.57815
Palladium electrodes replaced by $Pt_{1A(.08)}$ electrodes—aged 3 months										
1.0	0.72331	0.70180	0.69227	0.67083	0.66097	0.65168	0.63182	0.62098	0.60663	0.57734
2.0	.72361	.70195	.69237	.67103	.66111	.65176	.63199	.62119	.60724	.57812
5.0	.72388	.70221	.69266	.67125	.66132	.65208	.63214	.62148	.60761	.57841
6.0	.72391	.70223	.69271	.67137	.66140	.65217	.63233	.62149	.60761	.57843
7.0	.72395	.70227	.69277	.67137	.66136	.65215	.63237	.62153	.60767	.57848
19.0	.72449	.70298	.69340	.67196	.66199	.65291	.63288	.62197	.60821	.57905
20.0	.72453	.70296	.69347	.67203	.66199	.65290	.63297	.62212	.60837	.57907
Platinum electrodes replaced by $Pt_{1A(.1B)}$ electrodes—aged ½ to 1 hour										
1.0	0.72358	0.70173	(e)	(e)	(e)	(e)	0.63171	0.62099	0.60711	0.57800
2.0	.72467	.70297	0.69332	0.67200	0.66193	(e)	.63303	.62223	.60822	.57915
5.0	.72499	.70382	.69430	.67275	.66282	0.65382	.63399	.62299	.60911	.57998
6.0	.72520	(e)	.69449	.67307	.66171	(e)	.63423	.62329	.60932	.58002

[a] In the subscript describing the hydrogen electrode, the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parentheses refer to the current density at which the electrode was plated. For example, the electrode $Pd_{1B(.08)}$ was made of palladium sponge and was plated for 1 minute, using solution Pd_B at a current density of 0.08 amp/cm².

[b] First recorded emf 6 to 7 hours after the flow of hydrogen gas was started.

[c] Second recorded emf 52 hours later, after measurements at 0° to 20°C.

[d] Third recorded emf 107 hours later, after measurements at 0° to 60°C.

[e] Cell behaved erratically.

TABLE 6.—*Electromotive forces of the galvanic cell* Pd *or* Pt, $H_2(g)/H_2Ph(m_1)$, $KHPh(m_2)$, $KCl(m_3)/AgCl(s)Ag(s)$ *at 25° C for definite intervals of time after the flow of hydrogen gas was started*

[The hydrogen and the silver—silver-chloride electrodes were aged 3 months. Hydrogen electrodes were $Pd_{1B(.09)}$.][a]

	Molalities and pH values of the solutions									
$m_1 \times 10^4$...	16.373	28.010	29.096	47.034	49.281	62.049	73.487	100.00	143.56	180.00
$m_2 \times 10^4$...	8.1865	14.005	14.548	23.517	24.641	31.025	36.743	50.00	71.782	90.00
$m_3 \times 10^4$...	8.1865	14.005	14.548	23.517	24.641	31.025	36.743	50.00	71.782	90.00
pH.....	3.16	3.03	3.02	2.93	2.92	2.88	2.86	2.81	2.76	2.73
Time	Electromotive forces of the solutions									
hr	v	v	v	v	v	v	v	v	v	v
[b]	0.59284	0.57227	0.57085	0.55334	0.55170	0.54357	0.53779	0.52745	0.51565	0.50847
[c]	.59287	.57228	.57082	.55333	.55170	.54359	.53783	.57242	.51563	.50844
[d]	.59294	.57233	.57091	.55337	.55172	.54360	.53783	.52746	.51569	.50852
Palladium electrodes replaced by $Pt_{1A(.09)}$ electrodes—aged 3 months; last four, aged over a year										
1.0	0.59261	0.57207	0.57109	0.55345	0.55127	0.54378	0.53766	0.52730	0.51544	0.50839
2.0	.53000	.57251	.57109	.55382	.55175	.54360	.53788	.52754	.51574	.50853
5.0	.53018	.57272	.57124	.55404	.55194	.54374	.53800	.52772	.51595	.50866
6.0	.53022	.57273	.57133	.55406	.55199	.54378	.53800	.52776	.51596	.50873
7.0	.53029	.57283	.57139	.55415	.55202	.54380	.53813	.52777	.51595	.50878
19.0	.53091	.57339	.57180	.55481	.55279	.54445	.53866	.52856	.51650	.50927
20.0	.53098	.57351	.57197	.55481	.55281	.54447	.53877	.52856	.51658	.50927
Platinum electrodes replaced by $Pt_{5A(.13)}$ electrodes—aged from ½ to 1 hour										
1.0	0.53072	0.57334	0.57199	0.55488	0.55287	0.54423	0.53875	0.52856	0.51663	0.50900
2.0	.53161	.57422	.57276	.55545	.55332	.54513	.53970	.52900	.51699	.50998
5.0	.53263	.57508	.57360	.55627	.55422	.54600	.54056	.53007	.51784	.51061
6.0	.53267	.57517	.57371	.55631	.55428	.54622	.54067	.53008	.51788	.51098

[a] In the subscript describing the hydrogen electrode, the first numeral refers to the number of minutes the electrode was plated, the capital letter refers to the type of plating solution used, and the numbers in parentheses refer to the current density at which the electrode was plated. For example, the electrode $Pd_{1B(.09)}$ was made of palladium sponge and was plated for 1 minute, using solution Pd_B at a current density of 0.08 amp/cm².

[b] First recorded emf 4 to 5 hours after the flow of hydrogen gas was started.

[c] Second recorded emf 47 hours later, after measurements at temperatures from 0° to 20° C.

[d] Third recorded emf 99 hours later, after measurements at temperatures from 0° to 60° C.

IV. CONCLUSIONS

Reliable measurements can be made with hydrogen electrodes in aqueous solutions of acid potassium phthalate and phthalate solutions of pH ranging from 2.73 to 5.33 and for temperatures from 0° to 60° C.

The inconsistencies regarding the behavior of hydrogen electrodes in phthalate solutions reported in the literature are due to the type of hydrogen electrode used and not to the design of the cell, or to variations in the potential of the liquid junction formed between the solutions of phthalates and the potassium chloride of the calomel electrode, or to the unstable state or unaged condition of the electrodes, or to increased reduction of the phthalates at the hydrogen electrode because of impurities.

It has been found that the potentials of hydrogen electrodes prepared with palladium sponge of various thicknesses remain constant for 35 hours and increase only 0.3 mv after more than 100 hours of operation. Their characteristics are independent of the current density used in the electrolysis; of the concentration, composition, pH, and conductivity of the plating solution; and of the concentration, composition, pH, and buffer capacity of the phthalate solutions in which they are used.

On the other hand, the potentials of hydrogen electrodes prepared with platinum sponge increase steadily with time, and the rate of increase depends upon the thickness of the sponge. Frequently, such electrodes exhibit erratic behavior because of reduction of the phthalates at the electrode surface. Equilibrium values of the potential may be obtained at times if a very thin coat of platinum sponge is used and a large volume of solution is employed in the hydrogen-electrode compartment. However, the former condition cannot always be reproduced. The different behavior of platinum and palladium hydrogen-electrodes may be accounted for by the difference in their catalytic activity.

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EXTENT OF HYDROLYSIS OF STARCHES BY AMYLASES IN THE PRESENCE AND ABSENCE OF YEASTS [1]

By William Ward Pigman

ABSTRACT

The extent of enzymic conversion of corn starch, waxy maize starch, and potato amylose into materials fermentable by yeasts was studied for a number of different types of amylases. When the amylases are allowed to act on the starch substances, complete conversion to fermentable material may take place when the yeasts are allowed to act in the presence of the enzymes but not when the amylases and yeast act separately. The amylases that are capable of bringing about complete conversion are the cereal α-amylases and the fungal amylases. Certain bacterial amylases, pancreatic amylases, and the cereal β-amylases convert starch only partially to fermentable substances in the presence of yeasts. The experiments described in the present paper were carried out at starch concentrations comparable to those employed in industrial grain-alcohol processes.

With the notable exception of the malt amylases and the β-amylases, all of the enzyme preparations studied are capable of synthesizing unfermentable substances from maltose and presumably from starch hydrolysis products. For certain of the enzyme preparations, this synthesizing action provides an explanation for the lack of complete conversion of the starches to fermentable materials. For other enzymes, however, other explanations are more probable. The nature of the actions of the amylases are considered in relation to the structures of the starch substances.

Improved procedures are given for the preparation of soybean β-amylase and of potato amylose. Methods are described for bringing starches into solution without preliminary gel formation.

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I. INTRODUCTION

The action of amylases on starches is a fundamental process involved in many industrial operations, such as the preparation of alcoholic beverages and alcohol from grains and of dextrins for sizes and adhesives. Much experimental work has been carried out with the purpose of elucidating the nature of the action of the amylases. However, in most of this earlier work, the course of the enzymic hydrolysis has been followed by the measurement of the reducing power (usually calculated as maltose) during the reaction. In the experiments described in the present paper, the extent of hydrolysis brought about by the action of various amylase preparations on starch is measured by the amount of material present that is fermentable by baker's yeast. The determination of fermentable sugars, in many cases, appears to be of more value than reducing-sugar determinations, since the determination of the total reducing power of mixtures of dextrose, maltose, oligosaccharides, and dextrins obviously has little quantitative significance.[2]

The principal purpose of the present investigation is the determination of the ability of amylase preparations from different sources to bring about the conversion of starches to fermentable sugars. This study derives its importance from the necessity of knowing which types of amylases are the most suitable for application to the preparation of alcohol from starchy materials. In the course of obtaining the information necessary for the solution of the principal problem, considerable data has been obtained concerning the action of different types of amylases on starches.

In general, the experiments reported in the present paper were set up to simulate the commercial practice in the fermentation industries as far as possible. Thus, although most earlier investigators employed dilute solutions, the starch concentrations used in the experiments discussed below are of the order of 10 to 13 percent. In order to bring the starches into solution, the following general procedure was employed.

A stirred starch suspension was heated to 74° to 76° C and kept at this temperature for 10 to 15 minutes. The amylase preparation was added and allowed to act at 70° to 74° C for 10 to 15 minutes. (For the less heat-stable amylases, lower temperatures were employed in this stage.) Then, the stirred solution was heated at 100° C for 30 minutes, cooled to 45° to 50° C, and the final portion of the enzyme preparation was added. After several hours, the solution was allowed to cool to 30° C. Baker's yeast was added to the solution, and fermentation was continued for 5 days. The alcohol produced was determined by distillation and measurement of the specific gravity of the distillate. From the fermentation efficiency, as determined from blank experiments with maltose and dextrose and from the amount of alcohol

[2] The ideal method would permit the determination of the various constituents selectively, but the problem is very difficult. However, selective methods are available. (See for example: A. S. Schultz, R. A. Fisher, L. Atkin, and C. N. Frey, Ind. Eng. Chem., Anal. Ed. **15**, 496 (1943); M. Somogyi, J. Biol. Chem. **119**, 741 (1937). Although the fermentation method is capable of quantitative expression, it is more time-consuming than reducing-sugar determinations. Also the question arises as to the nature of the products that are fermented. It is well known that dextrose and maltose are fermentable by ordinary yeasts. However the nature of other fermentable products is less certain. One of the possible constituents of such products is "isomaltose", which may be identical with 6-α-glucosyl-glucose, although its constitution has not been completely established; it may be a mixture of several products and is usually reported not to be fermented by yeasts. On the other hand Myrbäck (J. prakt. Chem. **162**, 29 (1943) reports that maltotriose (presumably 4-glucose 4'-(α-glucosyl)-α-glucoside) is fermentable.

formed, the amount of fermentable sugars produced from the starch can be calculated. Under good conditions, the probable error of the method should not exceed ±1 percent.

II. ACTION OF MALT AMYLASES ON DIFFERENT TYPES OF STARCHES

As a result of preliminary experiments, a laboratory procedure was developed whereby complete conversion of corn starch by barley malt to fermentable substances is achieved. The best conditions for this purpose are summarized above and given in more detail in the experimental part. As illustrated in table 1, wheat, potato, and waxy maize starches also are converted completely to fermentable sugars by the malt amylases in the presence of yeasts. The amount of alcohol obtained from Lintner soluble starch seems significantly smaller than that obtained from the equivalent weight of dextrose, and it appears that this acid-modified starch may contain a small amount of material that resists the action of the amylases.

In spite of their obvious importance to the alcohol industry, there are but few reports in the literature of experiments of the above type. Hopkins, Cope, and Green [3] were able to bring about only 85.9 percent production of fermentable sugars by the action of barley diastase and yeasts on soluble starches. However, their results are not in particular disagreement with those in table 1, since the barley diastase is principally β-amylase, whereas the germinated barley (malt) contains other enzymes.

TABLE 1.—*Action of malt on several starches in the presence of yeast*

Starch	Alcohol from 24.96 g of dry starch [a]	Conversion efficiency (% of theoretical)
	g	
Corn	12.78	101.9
Wheat	12.81	102.1
Potato	12.71	101.3
Waxy maize	12.54	100.0
Lintner soluble	12.07	96.2
Theory	[b] (12.54)	(100)

[a] Corrected for malt blank.
[b] For 88.5-percent fermentation efficiency, as determined from blank experiments with dextrose and with maltose and calculated from the Gay-Lussac equation.

III. ACTION OF SEVERAL TYPES OF AMYLASE PREPARATIONS ON CORN AND WAXY MAIZE STARCHES AND POTATO AMYLOSE

According to the present conception of the structure of the starch substances, most starches are mixtures of two types of components: amyloses which consist of long unbranched chains of glucose residues connected through alpha 1,4′-glucosidic linkages; and amylopectin which consists of similar chains with branches. Corn starch, for example, contains 21 percent of amylose and presumably 79 percent of amylopectin.[4] In the present series of experiments, corn starch was

[3] R. H. Hopkins, G. F. Cope, and J. W. Green, J. Inst. Brewing **39**, 487 (1933).
[4] F. L. Bates, D. French, and R. E. Rundle, J. Am. Chem. Soc. **65**, 142 (1943).

selected for study because it is one of the most important starches from the standpoint of the alcohol and starch industries and because its composition is fairly representative of the cereal starches in general. In addition, it was decided to study the behavior of waxy maize starch and potato amylose as representative of the individual starch fractions; it has been demonstrated,[4 5] that waxy maize starch is free of amylose and consists solely of the amylopectin constituent. The amylose was prepared from potatoes by the butanol-precipitation procedure of Schoch.[5]

These starch substances were treated with amylase preparations from different sources by the method summarized in the introduction. In one set of experiments, the fermentation was carried out in the *presence* of the active amylases, and in a second set the amylases were destroyed before the yeast was added. The results obtained are summarized in table 2.

TABLE 2.—*Action of amylase preparations on several types of starch substances*

Amylase source	Degree of conversion to fermentable substances (percentage of theoretical)				
	Corn starch		Waxy maize starch (amylopectin)		Potato amylose [c]
	Amylases *present* during fermentation	Amylases *absent* during fermentation	Amylases *present* during fermentation	Amylases *absent* during fermentation	Amylases *present* during fermentation
Barley malt	103	89	100	90	
Malt extract		88		91	(100)
Malt α-amylase	100	87	99	89	
Kaffir malt	[a] 99	[a] 87 [b] (66)	[a] 99	[a] 90 [b] (80)	
Aspergillus oryzae-A	98	73	98	77	98
A. oryzae-B		81	100	69	
A. oryzae-C	98	85			
A. niger	97	77			
Whole pancreas	77	47	77	49	74
Bacillus mesentericus	55	53	57	47	71
Wheat β-amylase	69	64	70	59	96
Soybean β-amylase	64	62	68	65	

[a] In the presence of added calcium chloride.
[b] In the absence of added calcium chloride.
[c] The results reported for the amylose are to be considered as only of a preliminary nature. Since the amylase contained some insoluble material, the value obtained by the action of malt extract was taken as indicating 100-percent hydrolysis. In addition, the results are less accurate than those obtained from the corn and waxy maize starches, because one-fourth of the usual quantities were used in the experiments. It is hoped that additional experiments of this character can be carried out with purified amyloses at a later time.

The results in table 2 show that only certain amylase preparations are capable of converting starches completely to fermentable substances in the presence of yeasts. Such preparations are those containing the so-called cereal α-amylases (barley and kaffir malts) and the *Aspergillus* amylases. Barley malt and the malt extract are known to contain not only α-amylases but also β-amylases. The malt α-amylase preparation was made by the heat-inactivation procedure of Ohlsson,[6] which is said to inactivate the β-amylase component (probably incompletely). According to Kneen,[7] kaffir malt (germi-

[4] T. J. Schoch, J. Am. Chem. Soc. **64**, 2957 (1942); E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson, J. Am. Chem. Soc. **65**, 1382 (1943).
[5] E. Ohlsson, Z. physiol. Chem. **189**, 17 (1930).
[6] E. Kneen, Wallerstein Comm. **6**, 101 (1943). The sample used was supplied through the courtesy of Dr. Kneen, whose cooperation in this matter is gratefully acknowledged.

nated *Sorghum vulgare*) contains an α-amylase and no β-amylase. If either the heat-treated barley malt or the kaffir malt is free of β-amylases, it appears that the β-amylases are not essential for the saccharification of starches to fermentable substances. It is also clear from these results that the Lintner method,[8a] which mainly evaluates the β-amylase activity, is not sufficient for the determination of the amylase activity of commercial diastases. The measurement [8b] of characteristics of the α-amylase action such as the liquefying property or the dextrinizing property would seem to be of more value than the measurement of the saccharifying property as performed in the Lintner method.

The amylases in pancreas and *Bacillus mesentericus* emulsins [9] do not appear to be able to break down starches completely to fermentable sugars in the presence of yeasts. However, for these enzymes the problem is complicated because the enzymic action proceeds best near neutrality (pH 6 to 8) where the fermentation of maltose by yeasts takes place with difficulty if at all. Numerous experiments were carried out in which the pH during the fermentation period was held between 5 and 6, and the results shown in table 2 seem fairly reproducible. It appears that these emulsins are incapable of completely converting starches to fermentable sugars in the presence of yeasts, at least from a practical standpoint. The reasons for this discrepancy and its practical significance will be considered in more detail in section IV.

According to the results outlined in table 2, wheat and soybean β-amylases are unable to break down starches completely to fermentable sugars. Amylose, however, appears to be practically quantitatively hydrolyzed. These results are in agreement with earlier studies of the action of the β-amylases. Measurements of the reducing power of the products obtained by extended action of β-amylases on whole starches have shown that the reaction stops at a degree of hydrolysis of about 60 percent.[10] The fermentable sugar is maltose, and the unfermentable material is a dextrin with a high molecular weight. The straight chain amylose, however, is reported to be completely hydrolyzed to maltose by the β-amylases.[11]

For the soybean β-amylase (see table 2), there seems to be very little difference between the hydrolytic action in the presence and in the absence of yeasts. In the case of the wheat enzyme, however, there seems to be a significantly greater production of fermentable sugar when the reaction takes place in the presence of yeasts. The origin of this difference in action is unclear, but it seems probable that it may be due to the presence of small amounts of an α-amylase in the wheat preparation.

The results obtained for the β-amylases are important in relation to the proposed use of wheat extracts as diastatic agents for the conversion of wheats to fermentable substances. Wheat extracts are

[8a] For a discussion of the various modifications of the Lintner method, see, C. A. Browne and F. W. Zerban, Sugar Analysis, p. 1154 (John Wiley & Sons, Inc., New York, N. Y. 1941).

[8b] See: R. M. Sandstedt, E. Kneen, and M. J. Blish, Cereal Chem. **16**, 712 (1939); W. R. Johnston and S. Jozsa, J. Am. Chem. Soc. **57**, 701 (1935).

[9] As previously explained, the term "emulsin" is used in a general sense in place of "enzyme preparation," and refers to the crude or partially purified enzyme preparations from plants or animals. See, W. W. Pigman, J. Research NBS **30**, 159 (1943) RP1526 (footnote 2). B. Helferich and F. Vorsatz, Z. physiol. Chem. **237**, 254 (1935).

[10] See, for example, J. Blom, A. Bak and B. Braae, Z. physiol. Chem. **241**, 273 (1936); M. Samec and M. Blinc, Kolloid-Beihefte **49**, 93 (1939).

[11] A. R. Ling and D. R. Nanji, J. Chem. Soc. **127**, 629 (1925); K. H. Meyer, W. Brentano and P. Bernfeld, Helv. Chim. Acta **23**, 845 (1940).

known[12] to be low in α-amylase (liquefying) activity as compared to malted grains.

Although the wheat extracts are rich in β-amylase, the data of table 2 indicate that they are unsuitable for industrial starch conversions. If used, it would be necessary to supplement their action by the addition of malt or fungal amylases. Since wheat extracts have a high saccharifying power, it is again obvious that the Lintner determinations have little value for the evaluation of diastatic activities for the fermentation industries.

When fermentation of the products of amylase action takes place in the absence of amylases, complete conversion to fermentable sugars does not result (see table 2). Additional experiments, described and discussed in more detail in section IV, indicate that there is no single reason for the lack of complete hydrolysis in the absence of yeasts. For the fungal, bacterial, and pancreatic emulsins, incomplete hydrolysis is probably to be ascribed to the synthesis of unfermentable material during the period of diastatic action. For the β-amylase preparations, a residual high molecular weight dextrin is formed which is unattacked by both the β-amylases and the yeasts. Finally, for the cereal α-amylase preparations, it appears that an equilibrium may be established during the amylase action and that about 10 percent of unfermentable material is in the equilibrium solution.

The preparations from barley and kaffir malt carry the conversion of starches to 90 (87 to 91) percent of the theoretical quantity of fermentable sugar. This figure is appreciably higher than the value usually accepted for the extent of conversion as measured by reducing sugar determinations. (See footnote 10, page 109). In contrast to earlier results, there also appears to be no appreciable increase in the degree of conversion for the preparations which are known to contain large amounts of β-amylases (barley malt). As mentioned in the introduction, much of this difference between the results obtained from fermentable sugar and reducing sugar determinations is undoubtedly to be ascribed to the lack of quantitative significance of the reducing-sugar method when applied indiscriminately to mixtures of maltose, dextrins, and dextrose, such as are known to be obtained as the result of the action of the α-amylases. It should be noted, however, that recent work of Hopkins and Kulka[13] shows that malt α-amylase converts starches to reducing sugar (as maltose) to an extent of about 90 percent.

Although corn starch is reported to contain about 23 percent of the amylose and waxy maize starch to consist solely of amylopectin, the extent of action of the amylases on the two materials appears (within the experimental error) to be the same. If the amylopectin fractions of the two types of starches are identical, it would be expected that the corn starch would exhibit higher conversion by β-amylases to fermentable material because of its amylose content. However, the identity of the two amylopectin fractions has never been demonstrated.

In the first experiments carried out with the kaffir malt, complete conversion to fermentable substances was not achieved. It was found that the presence of calcium ions[14] was necessary for complete sac-

[12] H. C. Gore and S. Józsa, Ind. Eng. Chem. **24**, 102 (1932).
[13] R. H. Hopkins and D. Kulka, Wallerstein Comm. **5**, 115 (1942).
[14] One-half gram of calcium chloride was used in these experiments; this quantity corresponds to a concentration of about 0.2 g/100 ml.

charification. It is well known[15] that calcium salts protect certain enzymes from inactivation; and it would appear that the calcium salts probably function in this manner in the experiments carried out with the kaffir malt. The influence of salts (calcium and also sodium chloride) was investigated in connection with the action of the enzymes which were not capable of completely breaking down the starch to fermentable substances, but the kaffir malt was the only preparation exhibiting any appreciable effect on the degree of conversion.

IV. SYNTHESIZING ACTION OF AMYLASES ON MALTOSE

In the early stages of the present investigation the action of mixtures of several amylase-containing emulsins on starches was studied for the purpose of decreasing the amount of unfermentable material produced in the absence of yeasts. Surprisingly enough, it was observed that frequently less fermentable material was produced by the joint action of two amylase preparations than occurred when either acted alone. Since the most probable explanation for this behavior appeared to be that unfermentable material was being synthesized from the products of diastatic hydrolysis, experiments were set up in order to investigate the action of the enzyme preparations on dextrose and maltose.

For the study of the synthesizing action of amylase preparations on maltose and dextrose, two types of experiments were carried out with 15-percent solutions of the sugars. In one group of experiments, the enzymes were allowed to act on the buffered sugar solutions for several days at 57° C. The solutions were heated to destroy the enzymes, and the amount of fermentable sugar present was determined by fermentation with yeast and measurement of the amount of alcohol formed. As blank experiments, the same procedure was followed, but additional quantities of the enzyme preparations were added with the yeasts so that the enzymes were present during the fermentation period. The results obtained are summarized in table 3.

None of the enzyme preparations studied exhibited any synthetic action on dextrose solutions. With the probable exception of the

TABLE 3.—*Synthetic action of amylase preparations on maltose and dextrose*

Amylase source	Fermentable material remaining after enzyme treatment			
	Dextrose		Maltose	
	Enzymes *present* during fermentation	Enzymes *absent* during fermentation	Enzymes *present* during fermentation	Enzymes *absent* during fermentation
	Percent	*Percent*	*Percent*	*Percent*
Malt			97	98
Malt extract			100	99
A. niger	100	98	96	57 to 72
B. mesentericus	100	99	76	65
A. oryzae-A	100	99		76
Pancreas			84	72
Wheat β-amylase				102
Soybean β-amylase				101
Kaffir malt				91

[15] For discussion of this influence of calcium ions and of the previous literature, see E. Kneen, R. M. Sandstedt, and C. M. Hollenbeck, Cereal Chem. 20 399 (1943).

kaffir malt, the emulsins made from cereals were also inactive with respect to maltose solutions. However, the enzymes in fungal (*Aspergillus*), bacterial (*B. mesentericus*), and pancreatic emulsins produced considerable unfermentable material from maltose. In the presence of the enzymes and yeasts, the material produced by the action of the fungal enzymes is reconverted into fermentable material, but that formed by the bacterial and pancreatic enzymes is only partially reconverted. It should be noted that the figures given in table 3 (except those near 100 percent) are subject to much variation in individual experiments, presumably because of the slow rate of attainment of equilibrium conditions.

As far as is known, the synthesizing action of amylase preparations on maltose has not been previously recorded. It is interesting that similar action does not take place for dextrose solutions, particularly since most of these enzyme preparations contain α-glucosidases (maltases). In another respect, however, this synthesis is not surprising, since there are reports of the synthesizing action of bacteria, molds, and the corresponding enzyme preparations on sugar solutions. Thus, Tarr and Hibbert [16] have reported numerous examples of the synthesis of dextrans and levans by such agents. Also this synthesizing action undoubtedly is the explanation for the results of Stark,[17] who has studied the composition of the products of the action of pancreatic and salivary amylases on starch substances. According to the results which she reports, there is a continual shift in the nature of the products of hydrolysis, and there are more unfermentable dextrins in the later stages of the reaction than in the intermediary stages. As shown by the results in table 3, there can be little doubt but that the increase in dextrins observed by Stark is due to a synthesizing action by enzymes.

The fungal, bacterial, and pancreatic emulsins, which exhibit the synthesizing action, are mixtures of enzymes. As a result, it is impossible at the present time to ascribe the observed synthetic action only to the amylase components. The production of unfermentable material may be due to the amylases, but it also may be due to other enzymes, such as those which form the levans and dextrans from sugar solutions. In addition, it should be noted that these experiments were all carried out in the presence of phosphate buffers, and it is quite possible that the formation of unfermentable material may be due to the presence of phosphorylases, which are known to occur in many plant and animal extracts. It is hoped that it will be possible to continue this phase of the investigation in order that additional information may be obtained concerning the nature of the products synthesized and the enzymes which are responsible for the action.

V. APPLICATION OF BACTERIAL AND PANCREATIC EMULSINS FOR THE LIQUEFACTION OF CORN STARCH

If a 15-percent corn-starch suspension is heated at 74° to 78°C for a short time, a rigid gel forms. However, if a malt or a bacterial amylase preparation is added to the solution after it has commenced to thicken but before gelation has taken place, the solution rapidly thins; and even if the temperature is raised above 80°C, gel formation will not take place. This type of procedure has been employed in

[16] H. L. A. Tarr and H. Hibbert, Can. J. Research **5**, 414 (1931).
[17] I. E. Stark, J. Biol. Chem. **142**, 569 (1942).

industrial processes for the preparation of grain alcohol and is known as "premalting." However, as carried out industrially with malt, the process is rather inefficient, since the malt usually is mixed with the grains and then heated. The method used in the present work requires the addition of the liquefying amylase after the starch granules are swollen and partially dissolved and is a more efficient procedure. This method is the one described in the introduction and used in the experiments described in table 1. As shown there, when malt is used as the liquefying agent and subsequently as the saccharifying agent, complete conversion to fermentable substances takes place in the presence of yeasts.

The heat stability of certain types of bacterial amylases, notably those of *Bacillus mesentericus* emulsins, suggests their application in place of malt for the liquefaction of starches under such conditions that gelation will not take place. As previously noted, however, these enzymes are not suitable for the final saccharification of starches since the conversion is not complete in the presence of yeasts. It seemed possible that they might be applied as liquefying agents in conjunction with the use of malt or fungal amylases for the final saccharification. In order to test this possible application, corn starch was treated by the procedure outlined in the introduction except that bacterial amylases were used in the preliminary liquefaction stage. For the final conversion, malt or *Aspergillus* amylase was employed. In some experiments, the liquefying amylase was also present during the final hydrolysis, but usually it was destroyed by heat inactivation before the malt or fungal enzyme was added. In order to accentuate the action of the liquefying amylases, in certain experiments they were allowed to act on the starches for an additional several days at 57°C before they were destroyed and the saccharifying agent was added. Similar experiments were also carried out in which a pancreatic amylase preparation was used as the liquefying agent. The results are recorded in table 4.

TABLE 4.—*Action of amylase mixtures on corn starch*

[An amylase preparation is used for liquefaction of the starch. The solution is then heated at 100° C (enzyme destroyed.). The solution is then cooled to 45° C and one or more amylase preparations added. After several hours, the solutions are cooled and yeasts added.]

Amylases and conditions employed in experiments	Conversion to fermentable substances in the presence of yeasts (percentage of theoretical)
1. (a) *B. mesentericus* emulsin used for liquefaction. Malt usedfor final conversion.	91 [b]81
(b) *B. mesentericus* emulsin used for liquefaction. *B. mesentericus* emulsin added with the malt in the final conversion.[a]	91
(c) *B. mesentericus* emulsin used for liquefaction. *A oryzae* emulsin used for final conversion.	98 [b]90
2. (a) Pancreas emulsin used for liquefaction. Malt used for final conversion.	85 [b]77
(b) Pancreas emuslin used for liquefaction. Pancreas and malt present during final conversion.[a]	85
3. Pancreas and *B. mesentericus* emulsins used together in both stages.	[b]79

[a] A pH of about 5, as employed in the final conversion, is unfavorable for the action of the *B. mesentericus* and pancreas enzymes.

[b] The liquefaction period was extended to several days in order to provide good opportunity for synthesizing action.

It is evident from the results outlined in table 4 that the joint use of the bacterial emulsin in conjunction with malt amylases leads to an appreciable loss of fermentable material. This loss is particularly evident for the experiments in which the liquefying amylase remains in contact with the starch for a considerable time. Even for short periods of action there is an appreciable loss of fermentable substance. The *Aspergillus* emulsin, however, appears to have a greater ability than the malt in reconverting unfermentable material to fermentable substance. The results obtained for the bacterial emulsin are paralleled by those obtained by use of the pancreatic emulsin as the liquefying agent. It should be noted that the results in table 4 should be considered as representative results without much absolute significance; the values obtained will depend on the particular conditions selected for the measurements, since equilibrium conditions are not attained.

These experiments show that the application of bacterial and pancreatic agents for the liquefaction of starches may be a source of loss of considerable quantities of fermentable material, particularly if their action takes place for a considerable time, or if malt is employed as the saccharifying agent. Such agents would have to be used with considerable caution if employed for the preparation of grain alcohol. For other purposes, however, such as the preparation of sizes and adhesives, this behavior should not be objectionable and might even be advantageous.

VI. ACTION OF AMYLASE PREPARATIONS ON THE UNFERMENTABLE RESIDUAL DEXTRINS

In order to provide information concerning the unfermentable fraction produced by the various types of amylase preparations from starch, the action of enzymes on the residual dextrins was investigated. For these experiments, the enzymes were allowed to act on corn starch for several days at 57° C (first conversion), and the fermentable material was removed by yeasts, usually in the absence of the original amylases. The alcohol was distilled from the mixture, and the residue (which contained the unfermentable material) was treated again with an enzyme preparation (second conversion). Finally, yeasts were added, and the amount of fermentable substance formed by the action of the enzymes on the residual dextrins was calculated from the quantity of alcohol produced. The results obtained are summarized in table 5.

TABLE 5.—*Action of enzymes on unfermentable material produced from corn starch by malt, pancreas, and B. mesentericus enzymes*

First conversion		Second conversion	
Amylase preparation	Unfermentable material remaining	Amylase preparation	Unfermentable material remaining
	%		%
B. mesentericus	30	*B. mesentericus*	28
Do	32	*A. oryzae*	12
Do	45	*A. oryzae*	14
Do	46	Malt	20
Pancreas	19	Pancreas	19
Do	17	*A. oryzae*	17
Do	17	Malt	17
Malt extract	10	Malt (in absence of yeasts)	4
Do	10	Malt (in presence of yeasts)	4

Residual dextrins from action of malt amylases on corn starch.—As mentioned in the discussion of table 2, the malt enzymes are capable of converting starches to fermentable material to an extent of about 90 percent (in the absence of yeasts). This action was ascribed to the α-amylase constituent, since the same degree of conversion was attained by the use of the kaffir malt, which is reported to be devoid of β-amylase activity. The unfermentable material, by application of the procedure summarized above, was treated a second time with a malt extract subsequent to the removal of the fermentable material by fermentation. After the second treatment the enzyme was destroyed by heat inactivation. Considerable fermentable material was produced by this second enzyme treatment. In fact, the quantity was the same as that produced when malt enzymes and yeast were allowed to act together in the second treatment. These results indicate strongly the existence of an equilibrium between the unfermentable and the fermentable material. The equilibrium mixture appears to contain about 90 percent of fermentable and 10 percent of unfermentable sugars.

In the experiments previously mentioned (see discussion of table 3), the malt amylases were not able to synthesize unfermentable material from maltose. Apparently then, the equilibrium mixture produced from starch must contain fermentable materials, other than maltose, which are necessary for the establishment of the equilibrium. In agreement with this conclusion, it has already been established [18] that such mixtures also contain dextrose, trisaccharides, etc.

Residual dextrins from the action of bacterial and pancreatic emulsins on corn starch.—The fermentable material remaining from the action of *B. mesentericus* or pancreas emulsin on corn starch (after removal of fermentable material) was given a second treatment with the same enzymes. However, in neither case was any additional fermentable sugar formed. These results differ from those obtained by the action of malt enzymes on the unfermentable material resulting from the action of malt, as mentioned above. It would seem that the action of the bacterial and pancreatic emulsins does not involve a true equilibrium.

Although the unfermentable material produced by the action of pancreatic enzymes appears to be resistant to attack by both malt and *Aspergillus oryzae* enzymes, that formed by the bacterial enzymes is reconverted to fermentable substance to a considerable extent by both the malt and *Aspergillus* enzymes. The bacterial emulsins formed more unfermentable material than the pancreatic emulsins; it is of interest that after the secondary action of malt or *Aspergillus* enzymes, the total amount of fermentable material is approximately the same and is independent of the amylase type originally used (pancreas or *B. mesentericus* amylases). It does appear, however, that the *Aspergillus oryzae* enzymes are able to bring about a greater reconversion to fermentable substances than the malt enzymes.

VII. NATURE OF ACTION AND CLASSIFICATION OF THE AMYLASES

The results obtained in this investigation agree with the present views of the action of β-amylases. As mentioned in the discussion of table 2, the amount of fermentable material produced from corn and

[18] K. Myrbäck, J. prakt. Chem. **162**, 29 (1943); I. Stark, J. Biol. Chem. **142**, 569 (1942).

waxy maize starch by β-amylases appears to be the same in the presence or absence of yeasts; it also quantitatively agrees with the amount of reducing sugar obtained by most earlier workers and calculated as maltose. According to the accepted views, β-amylase action takes place by the successive removal of maltose residues from the ends of continuous straight chains of maltose residues. The action stops when some special type of linkage (other than the maltose alpha 1,4′ type, and possibly of the 1,6′ type) is encountered. The remaining material is believed to consist of a high molecular weight dextrin which resists the action of β-amylases and yeasts.

The nature of the action of liquefying amylases is less clearly established but is known to lead to the production of reducing dextrins mainly with 4 to 12 glucose residues per molecule. According to the previous work, the action must take place over or at the linkages which stop the action of the β-amylases, and there is no high molecular weight dextrin fraction. According to Hanes [19] and to Myrbäck,[20] the enzymic hydrolysis proceeds through the primary liberation of fragments containing about six glucose residues. These fragments subsequently may be broken down into shorter chains. The liquefying amylases which may operate in this manner are those which occur in pancreas, saliva, certain bacteria, fungus, and many cereal (particularly malted cereals) enzyme preparations. As a group, the amylases in these emulsins have been known as α-amylases.

The results obtained in the present investigation, discussed above in connection with table 2, indicate that there may be a fundamental difference in action between the various liquefying amylases. Thus, the cereal liquefying enzymes and the *Aspergillus* enzymes are able to break down starches completely to fermentable sugars in the presence of yeasts. The pancreas and *Bacillus mesentericus* enzymes, although they exhibit marked liquefying ability, do not appear to be able to accomplish the complete saccharification of starches to fermentable sugars in the presence of yeasts. It may be that the latter group of enzymes act similarly to the liquefying *Bacillus macerans* amylase. This enzyme is known to act with the production of cyclic hepta and hexasaccharides (Schardinger dextrins)[21]; but it is not established whether the formation of these materials is a primary action or is a result of secondary resynthesis from the products of hydrolysis. The synthesis of unfermentable materials from maltose by the pancreas and *B. mesentericus* enzymes suggests that it may be desirable to carry out such experiments with the *B. macerans* enzymes. However, until additional work has been carried out, it appears wise to separate the liquefying amylases into two main groups:

Group 1: Cereal α-amylases and *Aspergillus* amylases.

Group 2: Pancreas, and *B. mesentericus* amylases. Probably, also salivary and *B. macerans* amylases.

Until further information is available, it might be wise to reserve the term "α-amylases" for the liquefying cereal amylases.

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[19] C. S. Hanes, New Phytologist **36**, 101, 189 (1937).

[20] K. Myrbäck, J. prakt. Chem. **162**, 29 (1943).

[21] D. French and R. E. Rundle, J. Am. Chem. Soc. **64**, 1651 (1942); W. S. McClenahan, E. B. Tilden, and C. S. Hudson, J. Am. Chem. Soc. **64**, 2139 (1942); F. Schardinger, Zentr. Bakt. Parisitenk. [2], **22**, 98 (1908); R. W. Kerr, J. Am. Chem. Soc. **64**, 3044 (1942); E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson **65**, 1380 (1943).

VIII. EXPERIMENTAL PROCEDURE

I. MATERIALS USED

(a) ENZYME PREPARATIONS (EMULSINS)

The *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus mesentericus* and whole pancreas emulsins were active commercial preparations supplied through the courtesy of the Wallerstein Laboratories (New York, N. Y.), the Schwarz Laboratories (New York, N. Y.), and the Rohm & Haas Co. (Philadelphia, Pa.). Their properties have been previously described.[22]

The malt extract, wheat β-amylase and malt α-amylase preparations were special emulsins supplied through the courtesy of the Wallerstein Laboratories. The malt extract and wheat β-amylase have been previously described and were made by extraction and salt precipitation of the extracts. For the preparation of the malt α-amylase, the heat inactivation procedure of Ohlsson [23] was used. It is known that such treatment does not completely remove all β-amylase. The kaffir malt (germinated *Sorghum vulgare*) was supplied through the courtesy of Eric Kneen, of the University of Nebraska. According to the literature,[24] it contains only an α-amylase. The malt sample employed was a commercial (Pabst) distillers' barley malt with a Lintner value of 188° (dry basis).

Soy bean β-amylase was prepared by the general procedure of Newton and Naylor.[25] Palmetto and Missoy varieties were employed and were supplied through the courtesy of W. J. Morris of the Bureau of Plant Industry (U. S. Department of Agriculture, Beltsville, Md.). Several improvements [26] in the published procedure have simplified the process and have increased the yields. Best results were obtained by the following procedure:

The ground beans are placed in a percolator and extracted with acetone until the extracts are colorless. The extraction requires about 2 days, and the rate of flow is about 3 drops per second. The defatted beans are then allowed to dry at room temperature. From 1,800 g of original beans, about 1,300 g of defatted meal are obtained.

For the extraction of the β-amylase, 800 g of the defatted beans is treated with 3,200 ml of 50-percent ethyl alcohol containing 1.6 g of sodium sulfite. (The solution is made by mixing 1,700 ml of 95-percent ethyl alcohol, 1,530 ml of water, 1.6 g of sulfite, and enough glacial acetic acid to bring the pH to about 6 or 7.) The mixture is allowed to stand for 3 hours at room temperature, and during this time it is shaken at frequent intervals. The solution is filtered through coarse paper on a Büchner funnel; the residue is washed with about 500 ml of 50-percent alcohol; and the washings are added to the original extracts. The extracts are placed in a glass jar (surrounded by an ice bath and equipped with a mechanical stirrer) and cooled to 0° C. Enough 95-percent alcohol is added to the stirred solution (slowly from a separatory funnel) to bring the total alcohol concentration to 65 percent by volume. (If the above volumes have been used, 1,870 ml of 95-percent alcohol may be added.) The precipitated material is allowed to settle for 15 to 30 minutes. The supernatant liquid, which contains but little active β-amylase, is poured off and discarded. The gummy precipitate is covered with acetone for a few minutes. The acetone is poured off and more added. (Pouring off and not filtration is recommended at this stage as the acetone extracts filter very slowly.) This process is repeated several times and the gum, which rapidly hardens, is powdered with the aid of a spatula The powder finally is separated by filtration, and after removal of the acetone it is kept over calcium chloride in

[22] W. W. Pigman, J. Research NBS **30**, 159 (1943) RP 1526.
[23] E. Ohlsson, Z. physiol. Chem. **189**, 17 (1930).
[24] E. Kneen, Wallerstein Comm. **6**, 101 (1943).
[25] J. M. Newton and N. M. Naylor, Cereal Chem. **16**, 71 (1939).
[26] The assistance of Mary Grace Blair in carrying out this preparation is gratefully acknowledged.

a refrigerator. The yield of material ranges from 0.9 to 1.2 percent of the weight of the defatted beans. Newton and Naylor reported yields of 0.3 to 0.5 percent by their original procedure. The activity of the various preparations obtained by the above procedure fell between the limits 2,400 to 3,400[5] *L*.[27]

(b) STARCHES

The commercial corn starches used in the present investigation were supplied by the Clinton Co. (Clinton, Iowa) and the Corn Products Refining Co. (Argo, Ill.). The wheat starch was an experimental sample prepared from Rex, soft, white wheat and supplied through the courtesy of Cecil T. Langford, of the Northern Regional Research Laboratory (U. S. Department of Agriculture). The potato starch was a commercial sample provided by the Corn Products Refining Co., and the samples of waxy maize starch originated from the National Starch Products (New York, N. Y.) and the American Maize-Products Co. (New York, N. Y.). Merck "soluble starch according to Lintner" was purchased.

The procedure of Schoch (see reference on page 108) was employed for the preparation of the amylose from potato starch. By the introduction of certain minor changes it was possible to simplify the procedure somewhat; in the modified procedure, filtration of the amylase is possible rather than centrifugation. The procedure used follows:

A stirred suspension of 40 g of starch in 2 liters of water contained in a beaker is heated to boiling. After 400 ml of *n*-butanol is added, the beaker is placed in an autoclave and heated for 2 hours at a pressure of 20 lb/in^2. The autoclave is opened as soon as possible, and a mixture of 100 ml of butanol and 100 ml of isoamyl alcohol is added. The beaker is removed, covered with a watch glass, insulated carefully by wrapping well with a thick layer of cloth, and allowed to cool slowly to about 50°C for 24 to 48 hours. Subsequently, the temperature is allowed to drop to room temperature over a period of 24 to 48 hours. The rate of cooling to 50°C appears to be quite important; if it is too rapid the amylase fraction will appear as a gelatinous layer rather than as fine but discrete particles. The amylose fraction may be separated easily by filtration through a Buchner funnel covered first with a piece of filter cloth and then with a piece of hardened paper. However, the suspensions frequently contain a small quantity of gelatinous material which may cover the paper and prevent filtration. To avoid this difficulty, it is necessary to remove the interfering material. Since most of it appears in the original suspension as a scum between the aqueous phase and the supernatant alcohol phase, it should be carefully removed by decantation or by skimming with a spoon. In any case, if the filtration becomes too slow, it may be hastened by occasional scraping of the filter surface with a spatula. When the most of the mother liquor has been removed from the crystals, the wet mass is washed well several times with butanol-saturated water and finally several times with methanol.

The moisture content of the various starches were determined by drying them to constant weight in a vacuum at 90°C over calcium chloride. The values lay between 11 and 13 percent for waxy maize and corn starch; that for the soluble starch fell in the range 8 to 10 percent. The amylose had a moisture content of 2.5 percent. In all of the experiments in which these materials were employed for quantitative determinations, allowance was made for the moisture content A few experiments were carried out with methanol-extracted starches, but as no significant difference from the commercial starches could be observed, the commercial products were employed.

2. PROCEDURES

In preliminary work, numerous experiments were carried out in order to establish the best conditions for the liquefaction and sub-

[27] Cereal Laboratory Methods, Am. Assn. Cereal Chemists, Lincoln, Nebr., 1941.

sequent saccharification of the starches. Because of the variable heat resistance and optimal pH of the enzymes, no single procedure could be used. However, the procedures given below can be employed in most instances or can be modified slightly to provide the best conditions. The general applicability of these procedures is demonstrated by the production of alcohol in theoretical yields. Theoretical, in this case, means the amount of alcohol that is formed from an equivalent quantity of dextrose under the same conditions. Considerable work was carried out in order to determine the best conditions for the fermentation of maltose and dextrose These experiments are described in another publication,[28] in which it is shown that under the conditions employed here, the presence of nutrients have no influence on the alcohol yields and that the fermentation efficiency as calculated from the Gay-Lussac equation is close to 88.5 percent for dextrose and for maltose.

PROCEDURE FOR RELATIVELY HEAT-STABLE ENZYMES WHICH OPERATE WELL AT A pH OF ABOUT 5.0

Liquefaction step.—Weigh out accurately 24.96 g of starch (dry basis) into a 500-ml round-bottomed flask [a] (see notes below.) Add 125 ml of water and 40 ml of buffer solution [b] (p H, 5.2). Shake flask until all the starch is suspended and not clumped. Place flask in a water bath,[c] and stir contents with the aid of mechanical stirrer. Heat water bath to 74° to 76°C and keep at this temperature until the solution thickens appreciably or for about 10 minutes. Add a slurry of 0.5 g of malt extract (or 2 g of ground barley malt) in 10 ml of water to the flask, and use a little water to wash the enzyme preparation into the flask. Remove flask from bath and shake gently with a rotatory motion in order to work the enzyme into the partially pasted solution. Replace in bath, and stir contents for about 10 minutes at a temperature of about 70° to 72°C. Again remove flask from bath, and inspect in order to make sure that no lumps are present, particularly on the sides of the flask. If lumps are found, work them off by gentle manual rotation of the flask and contents. Then, replace the flask in the bath and stir mechanically.

"Cooking" step.—Raise the temperature of the bath to 100° C and hold at this temperature for 30 minutes. Remove flask from bath, stopper with sterile cotton, and place in a new water bath in which the temperature is regulated between 45° and 50° C.

Conversion or saccharification step.—To the "cooked" starch solution at a temperature of 45° to 50° C, add 1.0 g of malt extract (or 3 g of ground malt). Keep solution in the bath for at least 1 hour. Then remove and allow to cool to 30° C or less.

Fermentation step.—To the enzyme-treated solution, add 5 or 6 g of compressed bakers' yeast and 1 to 2 g of sterile potassium citrate. Close the flask with a stopper carrying a glass tube which is bent so that the liberated carbon dioxide may be discharged under water. Allow the solution to ferment for 5 days at about 30° C, and during this time shake flask twice each day.

Determination of alcohol.—At the conclusion of the fermentation, remove stopper and wash any adhering liquid into the flask. Connect the flask to a condenser. Distill almost 100 ml into a 100-ml volumetric flask. Place the flask containing the distillate in a constant temperature bath and make carefully to volume. Weigh the flask and contents, calculate the density, and from proper tables calculate the yield of alcohol. For details of these calculations and precautions that must be exercised, see the previously mentioned paper.[28]

NOTES

(a) Round-bottomed flasks were found more desirable for this step than commercial Florence flasks because the neck diameters are larger. For routine work, it was found desirable to flatten the bottoms of such flasks so that they could be kept in the incubator without a supporting ring.

[28] M. G. Blair and W. W. Pigman (manuscript pending).

(b) The buffer solution was prepared by dissolving the following quantities of substances in water and making to a volume of 1 liter:

40 g $NH_4H_2PO_4$
8 g $K_2HPO_4.3H_2O$
28 g KH_2PO_4.

(c) As a water bath for the liquefaction and cooking steps, a pan of water heated with a gas flame was found to be satisfactory and to be capable of good temperature regulation.

Modifications of procedure.—Certain types of amylases are destroyed too rapidly at 70° C to be employed in the above procedure. To this group belong the fungal, pancreatic, and probably the soybean and wheat amylases. When it is desired to test these enzymes, the procedure need only be modified slightly in the liquefaction step. Thus, after the starch suspension has been allowed to partially gelatinize, it is cooled rapidly to 50° C, and the enzyme is added and allowed to act for 15 to 30 minutes at this temperature. Then the solution is heated to 78° over a period of about 15 minutes, and finally rapidly to 100° C.

Some variations in the procedure.—For some enzymes it is desirable to carry out the reaction at a lower acidity than is most favorable for the malt and fungal enzymes. Thus, the *Bacillus mesentericus* and the pancreatic amylases have their optimal pH near pH 6 to 7. It is not desirable to maintain the pH at 6 to 7 during the cooking procedure, however, as an appreciable loss of fermentable sugars would result. In the experiments described in the present paper, the liquefaction and cooking steps were carried out at a pH of about 5.2. For the bacterial and pancreatic enzymes, 17 ml of *N* potassium hydroxide solution was added with the enzyme in the saccharification step. This quantity of alkali brings the pH to about 7.0.

The slight liquefying power of β-amylase preparations necessitates extension of the saccharification period to about 24 hours. In order to prevent the growth of micro-organisms during this period, it may be desirable to maintain the temperature at 57° to 60° C. If the higher temperatures are used, a fresh portion of the β-amylase should be added with the yeast in the final fermentation step. Such solutions also tend to foam badly during the fermentation and during the subsequent distillation of the alcohol.

A number of experiments were carried out in which the amylase was destroyed before the yeast was added. For these experiments it was necessary to keep the solutions for a considerable period in the presence of the amylases; this was accomplished by extending the saccharifying period to several days. Originally, considerable difficulty was encountered in preventing the growth of molds and bacteria during this time. However, by keeping the temperature at 50° C for 3 to 6 hours and then raising it to 57° C and keeping it at 57° C, growth of micro-organisms could be prevented. Since some of these enzymes may be destroyed at 57° C, a fresh portion of the enzyme preparation was added each day during the saccharification step. After several days, the solutions were heated at 100° C for 15 to 30 minutes in order to inactivate the enzyme. Finally, the solution was cooled and the yeast was added.

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A PHYSICAL METHOD FOR DETERMINING RESIDUAL WATER AND OTHER VOLATILE MATERIALS IN PURE SUBSTANCES

By Frank W. Schwab and Edward Wichers

ABSTRACT

A method is described for determining small amounts of water and other volatile substances present as impurities in various organic and inorganic compounds. The impurity is separated by fusing the substance in a cell attached to a collecting system and allowing it to freeze slowly while the vapors are collected by pumping them into a trap of known volume, cooled by liquid air. When the trap is warmed to room temperature, the pressure of the vapor within it, if below saturation, together with the temperature and volume, determine the quantity of impurity in terms of moles and if its identity is known, in terms of weight. An auxiliary procedure permits the impurity to be isolated as a liquid, in a capillary container, and thus to be identified.

It was found that the current Standard Samples of benzoic acid (39f and 140) contain less than 0.002 percent of water and do not adsorb water on exposure to an atmosphere of high humidity. The method is further illustrated by the determination of residual solvents in acetanilide crystallized from benzene and from a mixture of alcohol and water, and of water present in potassium dichromate as entrapped mother liquor.

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I. INTRODUCTION

In connection with a study of the properties of benzoic acid relative to the use of its freezing point as a thermometric fixed point, it became necessary to determine the amount of water retained by the substance under various conditions. The method devised for the purpose is believed to have advantages of simplicity and sensitivity, and possibly of accuracy, over previously published procedures which might have been used. No attempt will be made here to review the extensive literature dealing with the determination of water in organic substances. Experience in determining the purity of benzoic acid

from its freezing range [1] had shown that water does not appear to form solid solutions with benzoic acid, although it is moderately soluble in the fused acid. It therefore appeared that any water retained by the crystalline acid must be held either by adsorption on the crystal surfaces or by inclusion within crystals produced from and aqueous solution or from fused benzoic acid containing dissolved water. It seemed possible, therefore, to separate water by melting and freezing the acid in an evacuated system, and collecting the vaporized water in a suitable manner. Because of the small amounts of water involved and because water was the only volatile impurity known to be present, the method of collection used was to pump the water vapor into a trap cooled with liquid air. Then the amount collected was determined by warming the trap to room temperature and observing the pressure of the vapor. From the known volume of the trap, together with the temperature and the pressure (if below the saturation pressure), the quantity of water could be directly calculated. If the pressure was at saturation or near it, the determination was repeated with a larger trap or a smaller weight of sample.

An auxiliary procedure was to condense the vapor from the trap, where it was first collected, in a small capillary tube in which its volume could be measured with the aid of a microscope. It was possible to measure some of the properties of the liquid, such as its freezing point and refractive index, and thus to identify impurities of uncertain composition or to estimate the composition of a mixture of two known volatile impurities. With these variations, the method becomes generally applicable to the determination of small amounts of volatile impurities in fusible substances which are stable at their melting points. If the substance itself has a significant vapor pressure, the problem is not so simple and involves special considerations similar to those described by Shepherd [2] in determining the purity of condensed gases.

II. APPARATUS AND PROCEDURE

The apparatus is shown in figure 1. A and B are two cells of nearly equal capacity (about 50 ml) attached through lubricated ground joints to the rest of the train. One of the cells (A) is the container for the sample and the other (B) provides a control or blank of the amount of water and carbon dioxide contained in the air or adsorbed on the walls of the cell at the time of the experiment. (1) is a three-way stopcock through which either A or B can be connected to the train, and (2) is a stopcock not needed or used in the work described in this paper, but which provides connection to other apparatus not shown. C is a differential manometer (normally, but not necessarily, filled with mercury) connected to the system through the stopcocks 3, 4, and 5. Stopcock 6 connects the system to a source of dry air, free of carbon dioxide. D is a trap whose internal volume, together with that of the adjacent leg of the manometer, is known. It is provided with a stopcock, 7, and is connected to the train by means of lubricated ground joints. Three traps, with capacities of 21, 66, and 121 ml, respectively, were used alternatively in the experimental work to

[1] F. W. Schwab and Edward Wichers, *Precise measurement of the freezing range as a means of determining the purity of a substance*, Temperature—Its Measurement and Control in Science and Industry, p. 256-264 (Reinhold Publishing Corporation, New York, N. Y., 1941); also see Frank W. Schwab and Edward Wichers, *Preparation of benzoic acid of high purity*, J. Research NBS **25**, 747 (1940) RP1351.

[2] Martin Shepherd, *A critical test for the purity of gases*, BS J. Research **12**, 185 (1934) RP643.

be described. Each contained a plug of glass cotton to prevent the escape of minute ice crystals formed when water vapor enters the chilled trap. E is a thin-walled capillary tube used, when desired, to collect the liquid impurity by distillation from trap D. F is another trap like D, but serves only as a guard tube to prevent access of moisture from the line leading to the vacuum pump. This line is also provided with a stopcock (8). The stopcocks were of 2-mm bore; the inside diameter of the tubing connecting the parts of the train was 3 to 4 mm.

To make a determination, assemble the apparatus as shown in figure 1, with both cells, A and B, empty. Cool the trap D with liquid air and evacuate the entire system to a pressure not higher than a few hundredths of a millimeter of mercury. Fill the system with air free of carbon dioxide and dried by passing it over phosphorus pentoxide or through a suitable trap cooled with liquid air, and evacuate again. Repeat this cycle two or three times to aid in the removal

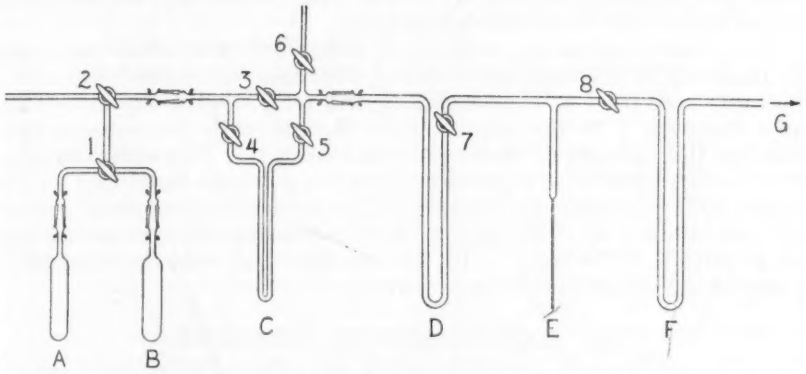

FIGURE 1.—*Apparatus for determining residual water and other volatile materials in pure substances.*

A, Cell for the sample; *B*, cell for control; *C*, manometer; *D*, trap of known volume; *E*, capillary container; *F*, trap; *G*, vacuum connection; 1, 2, 3, etc., 2 mm stopcocks.

of water adsorbed on the surfaces of the apparatus. Finally, fill the system again with dry air, close stopcock 1, and remove the two cells A and B. Handle the two cells as nearly alike as possible. Transfer a suitable weight of sample (usually 10 g) to A and reattach both cells to the train. Cool traps D and F with liquid air, open stopcock 1 to the cell containing the sample, lower the pressure in the system by several centimeters of mercury and maintain this reduced pressure during the next steps of the procedure by using the manometer to observe changes in pressure. Protect the lubricated joint of cell A with a suitable shield, warm the sample gently, and finally fuse it completely.[3] Let the liquid freeze slowly, add some heat with a flame, if necessary, to prevent rapid freezing, and further reduce the pressure in the system to complete evacuation. Let the pump run for several minutes with stopcocks 3, 4, and 5 open and with 6 closed. Then close cocks 3, 4, and 7. Let the trap D and the manometer warm to the temperature of the room. Examine the walls of the trap closely

[3] If the substance has a melting point too high to be fused in glass and the water or other volatile impurity is present as liquid trapped within the crystals, heating to a temperature high enough to insure thorough decrepitation of the solid can often be substituted for actual fusion.

for droplets of liquid and note whether the manometer reading indicates a pressure close to the saturation pressure of the liquid that is being determined. If the pressure is at or near saturation, repeat the determination with a smaller sample or with a trap of larger capacity. If the pressure is obviously below saturation, observe the pressure accurately and note the temperature of the room. From the temperature, pressure, and volume, calculate the molal quantity of the vapor. If the identity of the impurity is known, the calculation is made, if desired, in terms of its mass.

If the impurity is to be identified, evacuate the portion of the system between stopcocks 7 and 8, while cooling the guard trap F. Then close cock 8 and cool the capillary tube E with liquid air. Gently warm the parts of the train leading to E to promote the transfer of impurity into the capillary. When the transfer is complete, as evidenced by balance in the manometer, seal off the capillary tube. Some of the properties of the liquified impurity, e. g., its melting point, can be measured directly in this tube; others will require transferring the liquid to suitable apparatus.

When observations are completed, either before or after transfer of the impurity to the capillary receiver, evacuate the entire system with the same care used at the beginning and refill it with dry air. Then open stopcock 1 to the control tube B, and with the same technic measure the amount of water collected from B. The observed pressure is subtracted as a correction from the pressure developed by the vapors collected from the sample. This correction is normally small and can be omitted if the desired accuracy of the determination does not require it to be made. In the experimental work here reported, it amounted to about 0.04 mg of water.

III. EXPERIMENTAL RESULTS

To find the magnitude of errors in the determination of water which might be caused by the retention of water on the relatively large area of glass surface of the apparatus, known small amounts of water were introduced and determined. The water was provided in the form of sodium dichromate dihydrate. Well-formed crystals of this salt were broken, and tiny fragments from the inside of the large crystals were weighed and quickly transferred to the sample container. During this operation larger fragments of the salt showed no evidence either of deliquescence or efflorescence. The tiny crystalline fragments lost most of their water when the cell was heated to 100° C, but they were heated to fusion in accordance with the procedure given in the preceding section. From four samples of the salt weighing 3.66, 2.77, 1.70, and 1.29 mg, corresponding to 0.44, 0.33, 0.21, and 0.16 mg of water, respectively, the amounts of water recovered were 0.37, 0.26, 0.23, and 0.16 mg. In terms of 10 g samples used for the determination of water in benzoic acid, these results correspond to a maximum error of 0.0007 percent and an average error of 0.0004 percent. This apparent accuracy seemed sufficient for the purpose, but it might be improved by refinements of the apparatus and technic.

1. DETERMINATION OF WATER IN BENZOIC ACID

During the 30 years or more that benzoic acid has been used as a standard substance, there has always been some concern about its

possible hygroscopicity. Morey [4] suggested fusing the material so as to reduce, for a given weight of sample, the surface area on which adsorption could occur. Weaver [5] measured what he thought to be adsorption. He found, by titration, a decrease of 0.08 percent in the acidimetric value of a sample of benzoic acid that had been kept in a glass-stoppered bottle for about a year, and later detected water in it directly, although by a qualitative test. Świętosławski and coworkers [6 7] reported that they were able to detect as little as 0.001 percent of water in 15 g of benzoic acid, by an ebulliometric method. They further reported the water content of benzoic acid, Standard Sample 48a, issued by the National Bureau of Standards, as 0.0047 ±0.0008 percent. They also claimed that a pellet of benzoic acid, which contained 0.002 percent of water, had a water content of 0.012 percent after exposure in "a saturated atmosphere" of water vapor at 25° C for 3 hours. Jessup [8] was unable to detect the adsorption of water by the indirect method of determining the heat of combustion of benzoic acid before and after its exposure in an environment of high relative humidity.

The method herein described was used to determine the water content of two current Standard Samples of benzoic acid,[9] and also to learn whether these samples gain water when exposed to air of high relative humidity. Ten-gram portions of the acid, contained in cells like A and B in figure 1, were stored over a saturated solution of sodium carbonate decahydrate at about 23° C for 45 days. This saturated solution maintains a relative humidity of 90 percent at 23° C. It seems reasonable to believe that equilibrium between the crystals of the acid and the water in the air would be closely approached, if not completely realized, in the period named. Empty cells, to serve as controls, were stored with those containing the acid and were attached to the water-determining train simultaneously with them.

With the procedure described, the water contents of two 10-g samples of Standard Sample 39f not exposed to the humid atmosphere were 0.0015 and 0.0015 percent.[10] For two samples of the same acid, after exposure, the values were 0.0019 and 0.0019 percent. Single samples of Standard Sample 140 were similarly examined before and after exposure. Both results were 0.0014 percent. On the basis of these results, the water content of these Standard Samples of benzoic acid is negligible for the purposes for which the acid is normally used. Since the Standard Sample acid is known to contain not more than about 0.02 mole percent of nonvolatile impurities, it may also be concluded that the pure substance, in the condition of moderately fine crystals, is not significantly hygroscopic at ordinary temperatures in an atmosphere having a relative humidity not higher than 90 percent.

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[4] George W. Morey, *Benzoic acid as an acidimetric standard*, J. Am. Chem. Soc. **34**, 1027 (1912).
[5] E. R. Weaver, *The use of benzoic acid as a standard material*, J. Am. Chem. Soc. **35**, 1309 (1913).
[6] W. Świętosławski and S. Miernik, Bul. int. acad. polon. sci., Classe sci. math. nat. [A] 53–8 (1935).
[7] W. Świętosławski, M. Wojciechowski, and S. Miernik, Bul. int. acad. polon. sci., Classe sci. math. nat. [A] 59–64 (1935).
[8] Ralph S. Jessup, *Heat of combustion of benzoic acid, with special reference to the standardization of bomb calorimeters*, J. Research NBS **29**, 247 (1942) RP1499.
[9] 39f is a Standard for acidimetry and for calorimetry, and 140 is a Standard for microchemistry. Both are currently issued by the National Bureau of Standards.
[10] Since the determination was based on the pressure of the vapor, without identification of the volatile substance as water, this would be more strictly stated as 0.01 mole percent of volatile impurity, assumed to be water.

2. DETERMINATION OF BENZENE IN ACETANILIDE

In the course of purifying a quantity of acetanilide to be used as a microchemical standard, W. H. Smith and W. W. Walton of this Bureau crystallized the material from a solution in benzene. To determine the amount of benzene retained by the crystals, the procedure herein described was applied to two samples, one of the rather coarse crystals after drying in air and the other of a portion of these crystals which had been crushed to smaller size. Acetanilide melts at 113.4° C and has a relatively low vapor pressure at that temperature. The benzene was accordingly very easily separated from it. On the basis of the pressure in the trap, 0.11_2 percent by weight of benzene was found in the coarse crystals and 0.05_1 in the finer ones. The vapor collected in the trap was then condensed in the capillary tube. The freezing point of the liquid in the tube was about 5° C, which corresponds closely with that of benzene, and thus served to identify the volatile impurity.

3. DETERMINATION OF A MIXTURE OF WATER AND ALCOHOL IN ACETANILIDE

In the purification of another quantity of acetanilide, the material was recrystallized from its solution in a mixture containing about 20 percent (by weight) of water and 80 percent of ethanol. The crystals were crushed somewhat and dried under a reduced pressure of several mm of mercury at 50° C. The volatile constituents of a 20-g sample of these crystals were separated from the sample in the manner described and collected in a trap having a capacity of 121 ml. When the trap was warmed to about 25° C, droplets of liquid on its walls indicated that a condition of saturation existed. Thus, the observed pressure was the vapor pressure of a liquid presumed to be a mixture of alcohol and water. Since this vapor pressure is relatively insensitive to changes in composition of the liquid, it could not be used to determine the proportion of alcohol and water. The volatile material was condensed in the capillary tube, which was sealed and separated from the train. By measurement of dimensions with a microscope, C. P. Saylor, of this Bureau, determined the volume of the liquid as 0.015 cm^3 at 26° C. He also measured the refractive index, and from this measurement and the relation between the refractive index and the percentage of alcohol by weight, he interpolated the percentage by weight of ethanol in the mixture, and from this value the density on the assumption that the liquid contained only ethanol and water. The percentage of ethanol in the mixture (by weight) was found to be about 60 percent. The weight of the liquid, determined directly by weighing the capillary tube before and after emptying it, was 0.0155 g. This weight, together with the density calculated from the refractive index, yielded 0.017 ml as the volume, which is in satisfactory agreement with the volume determined from the measurements made with the microscope.

4. DETERMINATION OF WATER IN POTASSIUM DICHROMATE

Examination by C. P. Saylor of potassium dichromate intended as a standard for oxidimetry showed that the crystals contained droplets of liquid, presumably trapped mother liquor. Determination

of water in one sample of this salt, in accordance with the procedure herein described, yielded a value of 0.02_7 percent by weight. A second sample was at first not fused but kept just below the melting point (397.5° C), while the cell was being evacuated. Some decrepitation occurred when the salt was heated, but much more while it was cooling under reduced pressure. The crystals finally broke up into a powder. The water content thus determined was 0.01_9 percent. The sample was then fused, whereupon the value determined for the water content was 0.02_0 percent. The difference between this value and that for the first sample is believed to be considerably larger than the experimental error. It more probably represents nonuniformity in the samples. The complete elimination of water from potassium dichromate without melting the substance depends on a change of the crystalline form of the solid which occurs at a temperature of about 237° C. A similar behavior is not to be expected in the case of all crystalline substances, and the complete elimination of water from them without melting cannot be depended on.

During repeated fusions of potassium dichromate, a slight but persistent evolution of gas bubbles occurred. This gas was believe to be oxygen. The low pressure existing during the evacuation makes this method of detecting decomposition extremely sensitive, and since the volume of the gas evolved was small, it is evident that the amount of decomposition was very slight. Oxygen could not be held in the trap, hence it could not cause errors in the determination of water.

IV. DISCUSSION

From the illustrations which have been given, it will appear that the method is suitable for the determination of volatile impurities in a considerable variety of substances under the following conditions:

1. The impurity is insoluble in the solid form of the substance.

2. The impurity has at least a moderate vapor pressure, and the vapor does not depart significantly from the conditions of an ideal gas at room temperature, or at least at a temperature at which the trap and manometer can be uniformly maintained. Furthermore, the impurity also has a negligible vapor pressure at the temperature of liquid air.

3. The impurity is essentially a single substance of known molecular weight in the vapor state, and identifiable if its proportion by weight rather than its molal concentration is desired.

3a. If not a single substance, the components of the mixture must be known or identifiable unless it is sufficient to determine only their combined concentration.

The method is most readily adaptable to impurities in substances that are stable at the melting temperature, have a relatively low vapor pressure at that temperature, and an insignificant vapor pressure at room temperature.

Because of its relatively high sensitivity, the method is specially useful for such determinations as that of volatile impurities absorbed by crystals. For residues of solvents present as mother liquor entrapped within crystals, especially of inorganic salts, it has the advantage of a direct determination over the commonly used indirect method of observing the loss of weight on heating. Temperatures very much above the boiling point of water are often needed to set

free the solvent by rupturing the crystalline structure within which the tiny droplets of mother liquor are confined. When this rupturing does occur, in the phenomenon of decrepitation, the resulting mechanical losses invalidate the indirect method of "drying".

Within the limitations already given, the method can be applied to the analysis of liquid substances as well as solid substances, provided they solidify in well-formed crystalline structures when suitably refrigerated. Examples of liquids which have the requisite large difference in vapor pressure from that of the impurity being determined are, of course, much less common than those of substances which are solid at ordinary temperatures.

An attractive feature of the method is that its precision can be adjusted within rather wide limits by the degree of refinement with which the measurements of volume, temperature, and pressure are made. For a high degree of accuracy, it will be necessary not only to use exact physical measurements but to use a refined technic in separating and collecting the impurity and in obviating or correcting for errors caused by retention of the vaporized impurity on the surfaces of the apparatus.

Thanks are expressed to C. L. Gordon for preparing the drawing appearing in this paper.

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OPTICAL ROTATION AS AN INDICATION OF AROMATIC SUBSTITUENT INFLUENCES AND INTRAMOLECULAR INTERACTION [1]

By William Ward Pigman

ABSTRACT

As a result of a comparison of the rotations of certain aromatic β-glucosides and their tetraacetates, it is shown to be probable that the rotations of the acetylated glucosides are influenced by steric interactions between the aglycon group and the sugar portion of the molecule. This behavior is in contrast to the influences predominating in the case of the unacetylated compounds which, as shown in previous work, probably operate on the resonance of the aglycon aromatic ring or in an inductive fashion.

As a test for the explanation of the anomalous positive rotations of certain of the acetylated β-glucosides, the rotation of several of these substances in nitrobenzene solution was measured over the temperature interval 20° to 150° C. Over this temperature range, several of the glucosides, and in particular those which show anomalous positive rotations at room temperature, exhibit a change in sign of rotation from positive to negative.

The preparation and properties of the following new compounds are described: The *m*-nitrophenyl α-D-glucoside and its tetraacetate, the *o,p*- and *o,o'*-dinitrophenyl β-glucoside tetraacetates, and the *m*-nitrobenzyl β-glucoside tetraacetate. The rotatory dispersion of these and other glucosides is reported for five wavelengths over the visible spectrum.

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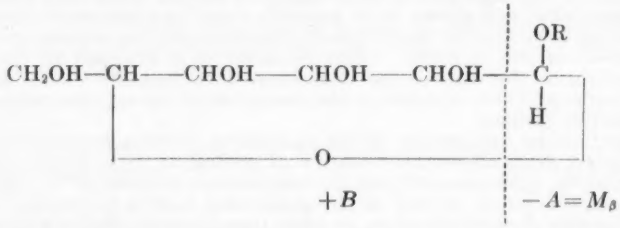
I. INTRODUCTION

It has been demonstrated [2 3 4] that the structures of the aromatic or alkyl radicals (the aglycon groups) of glycosides may be correlated

[1] Portions of this paper were presented before the Division of Sugar Chemistry and Technology of the American Chemical Society in Detroit, Mich., April 1943.
[2] C. S. Hudson, BS Sci. Pap. **21**, 254 (1926) S533.
[3] W. W. Pigman and H. S. Isbell, J. Research NBS **27**, 9 (1941).RP1399.
[4] L. C. Kreider and E. Friesen, J. Am. Chem. Soc. **64**, 1482 (1942).

with the optical rotations of the glycosides. Most of the correlations have been based on an ingenious method of comparison originated by Hudson,[2] in which the molecular optical rotation (molecular weight $\times[\alpha]_D$) is divided into two parts—that caused by carbon 1 and that caused by the remainder of the molecule. In a previous paper (see footnote 3), it was shown that the molecular rotations of the β-glucosides can be used directly in such correlations. The use of the molecular rotation of a glucoside rather than the partial rotation of carbon 1 is possible, since the application of Hudson's isorotation rules shows (see footnote 3) the existence of a direct proportionality between these quantities for a series of related β-glucosides. The reason for this proportionality appears to be that the primary influence of structural changes in the aglycon group (group RO in the formula) is exerted on the asymmetric carbon to which it is directly connected. Although the molecule contains other asymmetric centers,



variations in the structure of the group R have only a secondary and often negligible influence on the other centers. However, a marked difference in the influence of alkyl and aryl radicals on the partial rotations was noted; the aryl β-glucosides exhibit much more negative rotations than those of the alkyl β-glucosides, and both the partial rotations of carbon 1 (A) and of the remaining asymmetric carbons (B) are greater for the aryl than for the alkyl glucosides. Hence, it appears that the glucosides can be divided into two series—aryl and alkyl glucosides. The members of each of these series exhibit differences in the A values (partial rotation of carbon 1). However, the B value is the same for members in each of the series but differs for the two series.

In the previous work, the influence of structural changes in the aglycon group on the optical rotations of the acetylated β-glucosides was not given much consideration. One reason for not considering the acetylated derivatives further was that after a superficial examination it was quickly seen that a correlation of the optical rotations is much more difficult for the acetylated than for the unacetylated compounds. The present paper is an extension of the previous investigation to acetylated glycosides. As will be shown, these compounds in many instances exhibit quite different optical rotations from those shown by the unacetylated glycosides.

II. OPTICAL ROTATIONS OF ACETYLATED β-D-GLUCOSIDES

The molecular rotations of a number of acetylated β-glucosides are compared in table 1 with the rotations of the unacetylated β-glucosides. Many of these values have been taken from the literature, but it was

found necessary to prepare a number of new compounds and to remeasure the rotations of several more. The preparation of the new compounds and their properties are described in the experimental part of this paper.[5]

As noted in the previous paper, the aromatic β-glucosides have much more negative rotations than the corresponding alkyl glucosides. The value of −9,000 for the *n*-hexyl β-glucoside (8) [6] is typical of the rotations of the alkyl β-glucosides and that of −18,200 for the phenyl β-glucoside (1) is typical of many aromatic β-glucosides. The value for the benzyl β-glucoside (7), which has the aromatic nucleus removed from the glucosidic bond by a CH_2 group, is intermediate in character between the *n*-hexyl and the phenyl β-glucosides. The introduction of an additional CH_2 group gives phenylethyl β-glucoside (4), which has a rotation typical of that of the alkyl glucosides (8). Substitution of a nitro group in the aromatic ring of phenyl β-glucoside may take place in the *ortho*-, *meta*-, and *para*-positions, and each of the three possible compounds (5, 2, and 6) is considerably more levorotatory than the unsubstituted compound (1). It was shown in the previous paper that this influence of the nitro group (and of substituent groups in general, except some in the *ortho*-position) parallels the influence on the acidity of the corresponding phenols. It appears most likely that the influence of substituent groups is transmitted through the aromatic ring to the nearest asymmetric center (carbon 1). Evidence for the transmission of substituent effects through the chain is given by the near identity of the rotations of the *n*-hexyl and the phenylethyl β-glucosides (8 and 4); the intermediate values for the benzyl and *m*-nitrobenzyl β-glucosides (7 and 3) also agree with this interpretation.

It is evident from table 1 that the rotations of the β-glucosides in water are not paralleled by the rotations of the corresponding acetates in chloroform solution. Thus, the acetylated phenyl and the *n*-hexyl β-glucosides (9 and 18) have optical rotations of about the same magnitude; and the acetylated benzyl and *m*-nitrobenzyl β-glucosides (17 and 12) are much more levorotatory. The values for the nitrophenyl β-glucoside tetraacetates appear particularly anomalous when compared to those of the deacetylated compounds. Thus, although the *meta*- and *para*-isomers of the acetylated compounds (10 and 14) are more levorotatory than the unsubstituted compound (9), the *ortho*-isomer (15) not only is less negative but is dextrorotatory. There remains no doubt as to the value assigned to the *ortho*-isomer, for the original measurement by Glaser and Wulwek [7] has been checked recently by Montgomery, Richtmyer, and Hudson,[8] who also remark on its unusual positive sign.

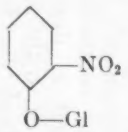
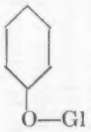
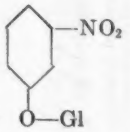
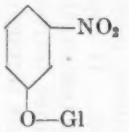
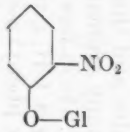
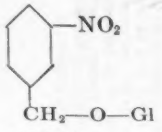
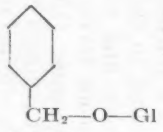
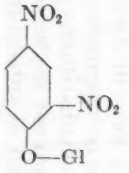
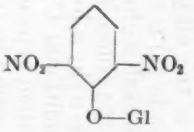
[5] The *o*-nitro and *p*-nitrophenyl β-glucoside tetraacetates were provided through the courtesy of Miss Montgomery and Doctors Richtmyer and Hudson, of the National Institute of Health.

[6] The numbers in parentheses refer to the compounds in table 1. The sources of most of the optical-rotation data are given in the previous publication, W. W. Pigman and H. S. Isbell, J. Research NBS **27**, 9 (1941) RP1399. In some cases more recent values from the following references have been used, E. Montgomery, N. K. Richtmyer and C. S. Hudson, J. Am. Chem. Soc. **64**, 690 (1942); W. W. Pigman and N. K. Richtmyer, J. Am. Chem. Soc **64**, 369 (1942); also N. K. Richtmyer, J. Am. Chem. Soc. **56**, 1633 (1934). A number of the compounds are new ones described in the experimental part of this paper. Although the *m*-nitrophenyl β-glucoside and its tetraacetate have been previously prepared, the rotations given in table 1 were measured during the present investigation.

[7] E. Glaser and W. Wulwek, Biochem. Z. **145**, 514 (1924).

[8] E. Montgomery, N. K. Richtmyer, and C. S. Hudson, J. Am. Chem. Soc. **64**, 690 (1942).

TABLE 1.—*Molecular and specific rotations of nitrophenyl β-D-glucosides, tetraacetates and related compounds*

Free glucosides in water (λ=589 mμ)				Acetates in chloroform (λ=589 mμ)			
	(1)		(5)		(9)		(14)
−18,200 (−71)		−31,900 (−106)		−9,300 (−22)		−19,200 (−41)	
	(2)		(6)		(10)		(15)
−26,800 (−89)		−31,000 (−103)		−17,400 (−37)		+21,100 (+45)	
	(3)		(7)		(11)		(16)
−16,700 (−53)		−14,600 (−54)		+18,000 (+35)		+137,000 (+266)	
	(4)		(8)		(12)		(17)

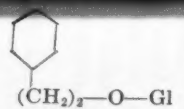
−16,700
(−53)

−14,600
(−54)

+18,000
(+35)

+137,000
(+266)

(4)



$(CH_2)_2—O—Gl$

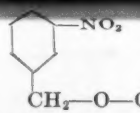
−8,800
(−31)

(8)

$O—Gl$

−9,000
(−34)

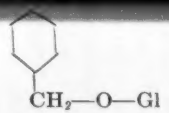
(12)



$CH_2—O—Gl$

−20,300
(−42)

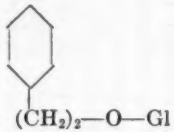
(17)



$CH_2—O—Gl$

−22,800
(−52)

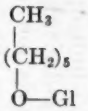
(13)



$(CH_2)_2—O—Gl$

−8,600
(−19)

(18)



$O—Gl$

−8,600
(−20)

Only very few β-glucoside tetraacetates have positive rotations, and apparently all known unacetylated β-glucosides are levorotatory. In addition to the *o*-nitrophenyl β-glucoside tetraacetate, the other previously known dextrorotatory compounds are the *ortho*-substituted chloro-, bromo-, and iodo-methylphenyl β-glucoside tetraacetates.[9] Because the effect appears to be limited to certain ortho isomers (compare compounds 10, 14, and 15), it was believed that the *ortho* effect might be accentuated in the corresponding di-*ortho*-substituted compounds. For this reason, the *o,o'*-dinitrophenyl β-glucoside tetraacetate (16) and the isomeric *o,p*-dinitrophenyl β-glucoside tetraacetate (11) were prepared. Deacetylation of these compounds without removal of the aglycon groups was not accomplished.

Both of the acetylated dinitrophenyl β-glucosides are dextrorotatory, and the di-*ortho*-isomer is more dextrorotatory than the known acetylated α-glucosides. Thus, *o*-nitrophenyl α-glucoside tetraacetate[10] has a rotation of $[\alpha]_D^{20}=+167$ ($CHCl_3$), and the *o,o'*-dinitrophenyl β-glucoside tetraacetate has $[\alpha]_D^{20}=+266$ ($CHCl_3$).

From the comparison made above, it is evident that the rotations of the acetylated β-glucosides are affected by influences different from those mainly operative in the case of the unacetylated isomers. As previously mentioned, the effect of the substitution of groups in the aromatic nucleus of phenyl β-glucoside appears to operate through the chain of atoms between the substituent group and the asymmetric center. It would appear to operate inductively or on the resonance of the benzene ring.[11] Since this type of influence is not prominent for the acetylated derivatives, it is more probable for these compounds that the substituent groups operate through space by virtue of their electrostatic charge or simply by steric effects of the type observed for diphenyl derivatives. The remainder of this paper will be devoted to a discussion of experiments which were carried out for the elucidation of the exceptional influence of the *ortho* groups.

III. NATURE OF THE "*ORTHO* EFFECT" OPERATIVE IN THE CASE OF THE ACETYLATED PHENYL β-GLUCOSIDES

1. SOLVENT EFFECT

If groups in the *ortho*-position exert their influence through a polarization of the asymmetric center at carbon 1 and by virtue of their electrostatic charge, it might be expected that the rotations would be strongly affected by the nature of the solvent. Consequently, the influence of the *ortho* groups should be much more noticeable when solvents with small dielectric constants are employed than when those with high dielectric constants are used; that is, the solvents with high dielectric strengths should be the best "insulators." The comparisons made in the preceding section support this explanation, since the solvent for the free glucosides is water (high dielectric constant, 80) and for the acetylated derivatives is chloroform (low dielectric constant, 5). Hence, it seemed desirable to carry out the rotation measurements

[9] A. Kunz, J. Am. Chem. Soc. **48**, 262 (1926).

[10] E. Montgomery, N. K. Richtmyer, and C. S. Hudson, J. Am. Chem. Soc. **64**, 690 (1942). Although the structures of these compounds and the configuration of the anomeric carbon atoms are not proved, the method of synthesis makes it probable that all are pyranosides with β-configurations.

[11] A number of summaries of the general types of influences exerted by substituent groups are available. See, for example: A. E. Remick, Electronic Interpretations of Organic Chemistry (John Wiley & Sons, Inc. New York, N. Y. 1943).

of the unsubstituted and the acetylated glucosides in the same solvent.

In table 2 are reported the results of measurements of the specific rotation of the *o*-nitrophenyl β-glucoside and its tetraacetate in a number of different solvents. Two solvents, acetic acid and dioxane, were employed for both the compounds. In these solvents, it will be noted that the rotation of the unacetylated compound remains levorotatory and the tetraacetate remains positive.

TABLE 2.—*Effect of solvent on rotation*

Solvent	*c* (g/100 ml)	$[\alpha]_D^{20}$	Solvent: dielectric constant (20°)	Solvent	*c* (g/100 ml)	$[\alpha]_D^{20}$	Solvent: dielectric constant (20°)
o-Nitrophenyl β-glucoside tetraacetate				*o*-Nitrophenyl β-glucoside			
$C_6H_5NO_2$	2.0	+19	36	H_2O	0.78	−105	80
CH_3COOH	1.0	+24	6	H_2O	2.0	−105	80
$CHCl_3$	4.0	+41	5	CH_3COOH	1.0	−91	6
Dioxane	1.0	+23	2	Dioxane	1.0	−125	2

Since acetic acid and dioxane have low dielectric strengths, the polarization influence of the *ortho* group should be much more pronounced for the acetic acid and dioxane solutions of the unsubstituted *o*-nithrophenyl β-glucoside than for aqueous solutions; as a result, the rotations in these solvents should be less levorotatory and possibly even be dextrorotatory as for the acetylated derivatives. Similarly, the rotations of the acetylated derivatives would be expected to be more levorotatory in the solvents of higher dielectric strength (i. e., nitrobenzene).

Actually, as shown in table 2, the rotations observed in the various solvents do not agree with these predictions. It is believed, then, that the anomalous rotation of the *o*-substituted derivatives of the acetylated β-glucosides is not due primarily to a polarization of the asymmetric centers by the groups in the ortho position.

2. TEMPERATURE INFLUENCE

Another explanation for the differences between the rotations of the acetylated and the unacetylated glucosides and in particular for the anomalous rotations of the acetylated *ortho*-substituted phenyl β-glucosides might be that esterification of the hydroxyl groups by the bulky acetyl groups would allow interaction between the acetyl and the aglycon groups. Such interaction might take the form of weak bonds between these groups, or it might operate through steric hindrance to free rotation of the aglycon group about the glucosidic linkage. Examination of models of the acetylated β-glucosides shows that interaction is very probable and should be accentuated by the presence of bulky groups in the ortho position.

It has been emphasized by earlier workers [12] that interactions between the various groups and solvent effects should decrease as the temperatue is increased and that measurements of the optical rotation at high temperatures tend to minimize these influences.

[12] K. L. Wolf, Trans. Faraday Soc. **26,** 315, 350 (1929); T. M. Lowry, Optical Rotatory Power, p. 349 and 353; Longmans, Green & Co., New York, N. Y., 1935. See also discussion by W. Kauzmann, J. Am. Chem. Soc. **64,** 1626 (1942).

At the higher temperatures, the vibrational energy of the molecules is greater and tends to disrupt weak bonds between groups in the molecule. Hence, measurements of the influence of temperature on the optical rotation should be of value in interpreting the influence of the structures of the aglycon groups of the acetylated β-glucosides on the optical rotations. For this reason, the optical rotations of a number of acetylated β-glucosides in nitrobenze solutions were measured at temperatures from 20° to 150° C. The details of these measurements are given in the experimental part of this paper. In general, they were carried out by dissolving the acetylated gluco-

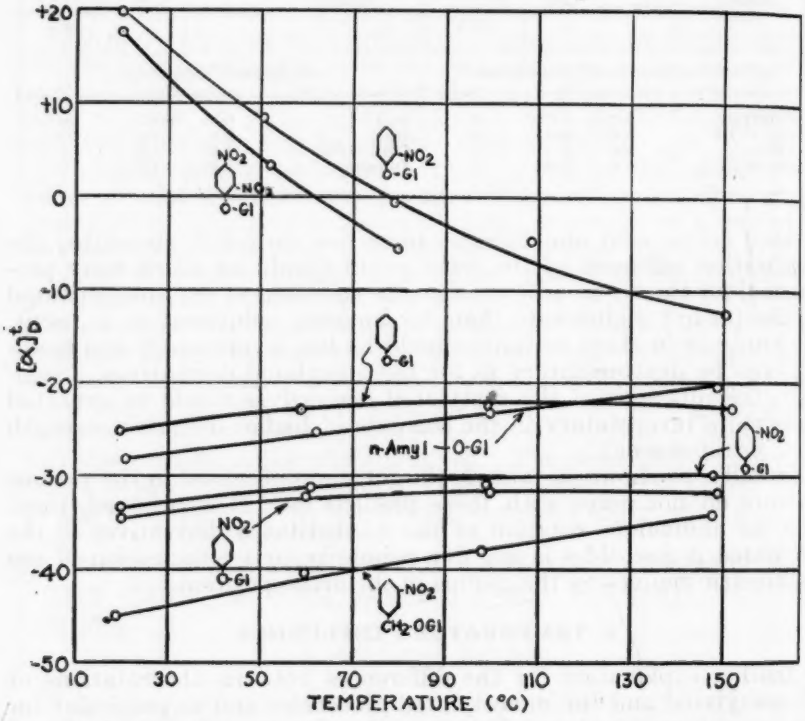


FIGURE 1.—*Influence of temperature on rotation of some β-glucosides.*

sides in nitrobenzene at room temperature and then by heating the solution. At various predetermined values, the temperature was held constant, and the optical rotation was measured. After the measurements at the highest temperature had been made, the solutions were cooled to 20° and reread in order to determine whether decomposition or evaporation had taken place. The observed rotations were corrected for the change in density of the solvent with temperature.

The influence of temperature on the specific rotations of several acetylated β-glucosides is illustrated in figure 1. The most noteworthy feature is the change of the sign of rotation of the *o*-nitrophenyl and the *o*, *p*-dinitrophenyl β-glucoside tetraacetates as the temperature increases. From the general direction of the curves it appears that

at sufficiently high temperatures the rotation of the *o*-isomer approaches those of the *meta*- and *para*-isomers not only in sign but also in absolute value. It is believed that these results provide strong evidence that the unusual positive optical rotations of certain of the *ortho*-substituted phenyl β-glucoside tetraacetates [13] are due to steric interactions between the groups in the *ortho*-position and groups attached to the asymmetric centers.

It appears that there is considerable interaction between the aglycons and the asymmetric centers of the *n*-hexyl and the *m*-nitrobenzyl derivatives, for the rotation of these compounds varies considerably as the temperature changes. At 150°, the rotation of the *n*-hexyl derivative becomes less negative than that of the phenyl derivative. It would seem that at the higher temperatures the predominating influence of steric effects is replaced by inductive and resonance influences of the same nature as those previously noted for the unacetylated glucosides.

The influence of temperature on optical rotation has been studied by a number of workers. However, instances of a reversal in sign as result of temperature changes are rather rare. Solutions of invert sugar [14] show a reversal in sign of rotation as the temperature is raised, but this example is not comparable with the results of the present investigation, since solutions of invert sugar contain a number of different substances. The inversion of rotation noted for solutions of tartaric acid (see footnote 14) malic acid and sodium malate [15] seems more likely to be of the same nature as that for the *o*-nitrophenyl β-glucoside tetraacetate.

IV. COMPARISON OF THE ROTATIONS OF THE PHENYL GLUCOSIDES AND OF THEIR TETRAACETATES

In the previous publication,[16] the partial rotations of carbon 1 (A) and of the sum of the other active carbons (B) of the glucosides and their tetraacetates were calculated by use of the isorotation rules of Hudson.[17] Since then, Montgomery, Richtmyer, and Hudson [18] have prepared the *o*-nitrophenyl α-glucoside and its tetraacetate, and in the present paper the *meta*-isomers are described. From the rotations of these compounds, the A and B values for two more pairs of glucosides and their acetates can be calculated, since the rotations of corresponding β-glucosides have been described previously. In table 3 are listed the $2A$ and $2B$ values for all of the known pairs of substituted-phenyl glucosides and the tetraacetates.

The $2A$ and $2B$ values for the *o*- and m-nitrophenyl glucosides and for the acetylated *meta*-derivative agree well with the values previously obtained for the aromatic β-glucosides. As was also pointed out by Montgomery, Richtmyer, and Hudson, the $2B$ value for the acetylated *ortho*-isomers is exceptionally large; on the other hand, the $2A$ value

[13] The optical rotation of the *o,p*-dinithropenyl β-glucoside tetraacetate could not be measured at temperatures exceeding 80° C because decomposition into unknown products takes place above this temperature. The *o,o'*-isomer was even more unstable and no measurements are reported. It should also be noted that for the other compounds there was no evidence of mutarotation, since no change of rotation could be observed during the period at the fixed temperatures.

[14] H. Landolt, Ber. deut. Chem. Ges. **13**, 2329 (1880).

[15] T. Thomsen, Ber. deut. Chem. Ges. **15**, 441 (1882).

[16] W. W. Pigman and H. S. Isbell, J. Research NBS **27**, 9 (1941) RP1399.

[17] C. S. Hudson, BS Sci. Pap. **21**, 241 (1926) S533; Rapports sur les Hydrates de Carbone, p. 59 (10th Conference of the International Union of Chemistry, Liege, 1930).

[18] E. Montgomery, N. K. Richtmyer, and C. S. Hudson, J. Am. Chem. Soc. **64**, 690 (1942).

is much smaller than that for the aromatic glucosides and is close to that for the aliphatic glucosides (the $2A$ value for the acetylated *n*-hexyl glucoside is 59,000). In view of the results obtained in the present investigation and discussed above, the exceptional rotations of the acetylated *ortho*-isomers most probably are to be ascribed to interaction between the groups in the *ortho*-position and the groups attached to carbons 2, 3, 4, or 5.

TABLE 3.—*Sums and differences of molecular rotations of alpha and beta pairs of substituted-phenyl glucosides and tetraacetates* [a]

D-glucosides	Not Acetylated		Acetylated	
	Rotations measured in H_2O for D line		Rotations measured in $CHCl_3$ for D line	
	Difference, $2A$	Sum, $2B$	Difference, $2A$	Sum, $2B$
Phenyl	67,800	31,400	80,600	62,000
p-Aminophenyl	[c] 70,300	[c] 35,000		
p-Hydroxyphenyl	65,900	31,300		
p-Benzoyloxyphenyl [b]			82,900	67,100
p-Nitrophenyl	95,800	33,800	113,100	74,700
m-Nitrophenyl	84,050	30,400	98,300	64,100
o-Nitrophenyl	94,000	30,100	57,300	99,500
		Avg 32,000		

[a] For origin of data, see footnote 6, p. 131.
[b] Incorrectly termed "*p*-Benzoylphenyl" in the first publication.
[c] One rotation measured in methyl alcohol.

V. MEASUREMENT OF THE ROTATORY DISPERSIONS OF SOME AROMATIC GLUCOSIDES AND THEIR TETRA-ACETATES

The variation of optical rotation with the wavelength of the light source used for the measurements (called rotatory dispersion) frequently may be expressed by a simple form of the Drude equation:

$$\alpha = \frac{A}{\lambda^2 - \lambda_0^2}, \tag{1}$$

where α is the rotation at the wavelength λ, A is the so-called rotation constant, and λ_0^2 is the dispersion constant. Sometimes the addition of a second term, B/λ^2, is necessary in order that the experimental data be satisfied completely. According to Lowry,[19] rotatory dispersions which fit the one-term equation are to be described as "simple rotatory dispersions"; those which require another term in the equation are to be known as "complex rotatory dispersions." A simple graphical method for testing the applicability of the equation 1 to rotatory dispersion data consists in plotting $1/\alpha$ vs. λ^2. If a straight line results, the dispersion is of the "simple" type.

According to equation 1, the rotation becomes infinite when $\lambda = \lambda_0$. The wavelength λ_0 sometimes is assumed to represent an optically active absorption band, which lies in the ultraviolet region for colorless substances, such as the sugars and their derivatives. The determination of the position of this absorption band is a matter of interest, since it may provide some information concerning the nature of the groups

[19] T. M. Lowry, Optical Rotatory Power, p. 123, (Longmans, Green, & Co., London, 1935).

that are responsible for the absorption and probably also for the optical rotation in the visible region. For this reason it appeared desirable to measure the rotatory dispersion of some of the compounds considered in the present paper.

TABLE 4.—*Equations for dispersions* ($T=18°$ *to* $21°$ *C*)

$$[\alpha]=\frac{A}{\lambda^2-\lambda_0^2}$$

Compound	Solvent	A	λ_0^2 (λ in microns)	λ_0 (angstrom units)
Phenyl β-glucoside tetraacetate	$CHCl_3$	−6.2	0.066	2570
o-Nitrophenyl β-glucoside tetraacetate	$CHCl_3$	−10.2	.072	2680
p-Nitrophenyl β-glucoside tetraacetate	$CHCl_3$	−9.6	.099	3150
o-Nitrobenzyl β-glucoside tetraacetate	$CHCl_3$	−12.6	.046	2140
o,o'-Dinitrophenyl β-glucoside tetraacetate	$CHCl_3$	+55.9	.137	3700
Phenyl β-glucoside	H_2O	−21.9	.050	2240
p-Nitrophenyl β-glucoside	H_2O	−25.8	.100	3160
o-Nitrophenyl β-glucoside	H_2O	−25.6	.058	2420
p-Nitrophenyl β-glucoside	H_2O	−28.3	.073	2700
p-Nitrophenyl α-glucoside	H_2O	+56.6	.048	2190
Some measurements previously reported for alkyl glucosides				
Methyl β-glucoside tetraacetate [a]	$CHCl_3$	−1.538	0.03	1730
Methyl β-glucoside [b]	H_2O	−6.135	.0250	1580
Methyl α-glucoside [b]	H_2O	+30.85	.0244	1560

[a] T. L. Harris, E. L. Hirst, and C. E. Wood, J. Chem. Soc. (1932) 2108.
[b] N. A. Sörensen and B. Trumpy, Z. physik. Chem. [B] 28, 135 (1935).

In table 4 are summarized the rotatory dispersion measurements that are of the simple type and that fit the one-term Drude equation 1. The measurements were carried out by use of a Schmidt and Haensch precision polarimeter; they were made at five wave lengths: 436, about 512, 589, and about 645mμ. The details are given in the experimental part of this paper. Data also are given in the experimental part for the *o*-nitrophenyl and the *o,p*-dinitrophenyl β-glucoside tetraacetates; these data could not be fitted to the simple form of the Drude equation and are of the "complex" type.

It is of interest that two of the *ortho*-substituted derivatives have "complex" rotatory dispersions. These same acetylated derivatives are the compounds that exhibit the anomalous positive rotations, as previously mentioned. Peculiarly enough, the *o,o'*-derivative, for which the effect might be expected to be greatly accentuated, exhibits a simple dispersion. The optical rotation of the acetylated *ortho*-substituted derivatives could not be measured by use of a saccharimeter and white light because of the difference in color of the two sides of the optical field. This difference in color undoubtedly arises from the disparity in the dispersion of quartz and of the acetylated *ortho*-substituted derivatives.

In table 4, the values of the dispersion constant (λ^2_0) are given. As previously mentioned, λ_0 may represent optically active absorption bands. It is of interest then that for the aromatic glucosides the position of these bands is closer to the visible region than for the alkyl glucosides and that the introduction of nitro groups into the aromatic nucleus produces a shift in the position of the bands still further toward the visible region. These values might be inter-

preted as indicating that the groups responsible for the absorption are alkyl groups (for the alkyl glucosides), the aromatic nucleus (for the phenyl glucosides), and the nitro or nitrophenyl groups (for the nitrophenyl glucosides). It is well known that absorption of ultraviolet light occurs for such groups in the approximate regions represented by the values of λ_0. The absorption bands (λ_0) for the acetylated derivatives, as a rule, lie closer to the visible region than those of the unacetylated derivatives. It would appear that the acetyl groups may have some influence on the position of the band; however, there is no evidence that the shift in position may not be a solvent effect.

VI. EXPERIMENTAL PROCEDURE

1. PREPARATION OF GLUCOSIDES

(a) SUBSTITUTED-PHENYL D-GLUCOSIDES

The new *beta*-isomers were prepared by reacting tetraacetylglucosyl bromide, the substituted-phenol, and sodium hydroxide in aqueous acetone solution at room temperature.[20] The *alpha*-isomers were obtained by a modification of the Helferich, Schmitz-Hillebrecht method, as described for *o*-nitrophenyl α-D-glucoside.[21] Deacetylation of the tetraacetates was accomplished by the action of small quantities of barium methylate in boiling methanol.[22] The successful deacetylation of the dinitrophenyl β-D-glucosides has not yet been achieved; under all of the conditions employed, the aglycon group was removed with the acetyl groups.

m-Nitrophenyl α-D-glucoside and tetraacetate.—The product obtained by fusion of pentaacetylglucose and *m*-nitrophenol with zinc chloride did not crystallize even when purified by fractional extraction. After deacetylation, a sirupy product was obtained. The material, which was soluble in isopropyl alcohol but which was not precipitated from the alcohol solution by the addition of absolute ether, crystallized, giving a small yield of a mixture of the *alpha*- and *beta*-isomers. (From 30 g of pentaacetylglucose, 3 g of the mixture was obtained, which was principally the *alpha*-isomer). The *alpha*-isomer was obtained in pure condition by crystallization from water until the rotation remained constant. The substance crystallizes from water apparently in the anhydrous condition as long slender needles. It was dried in high vacuum at 100° C before the physical properties were determined. It melts at 172° to 175° C (cor.); at 20° C, 1.6 g dissolves in 100 ml of aqueous solution. $[\alpha]_D^{20} = +189$ (c, 1.6; water). Analysis: Calculated for $C_{13}H_{17}O_8N$: N, 4.65; Found: N, 4.60.

Tetraacetate.—The glucoside was acetylated with sodium acetate and acetic anhydride and recrystallized several times from ethyl alcohol. It crystallizes as rectangular prisms. The pure material has a melting point of 104.2° to 104.4° C (cor.); $[\alpha]_D^{20} = +173$ (*c*, 2.4 $CHCl_3$). Analysis: Calculated for $C_{20}H_{23}O_{12}N$: N, 2.98; Found: N, 3.05.

o,p-Dinitrophenyl β-D-glucoside tetraacetate.—About 4.5 g of the tetraacetate was obtained from 34 g of tetraacetylglucosyl bromide by use of the directions of Glaser and Wulwek. Several recrystallizations from ethyl alcohol or acetone gave a colorless product crystallizing

[20] The original method is a modification of the Michael synthesis (Am. Chem. J. **1**, 305 (1879), but the specific directions given by E. Glaser and W. Wulwek, Biochem. Z. **145**, 514 (1924) were followed.

[21] E. M. Montgomery, N. K. Richtmyer, and C. S. Hudson, J. Am. Chem. Soc. **64**, 690 (1942); B. Helferich and E. Schmitz-Hillebrecht, Ber. deut. Chem. Ges. **66**, 378 (1933).

[22] H. S. Isbell, BS J. Research **5**, 1185 (1930) RP253. G. Zemplén and E. Pacsu, Ber. deut. Chem. Ges. **62**, 1613 (1929).

s elongated prisms and melting at 173° to 177° C (cor.); $[\alpha]_D^{20} = +34.5$ (c, 4; $CHCl_3$). When the rotation is read in a saccharimeter an appreciable difference in the tint of the two sides of the field is observed. However, no significant difference was observed in values measured with the saccharimeter from those obtained by use of a polarimeter. Analysis: Calculated for $C_{20}H_{22}O_{14}N_2$: N, 5.45; Found: N, 5.59.

o,o'-Dinitrophenyl β-d-glucoside tetraacetate.—From 34 g of tetraacetylglycosyl bromide and by the use of the same proportions of reagents as described by Glaser and Wulwek, about 20 g of the acetylated glucoside was obtained. After a number of recrystallizations from acetone, from which the compound crystallizes as truncated prisms, it was found to melt at 175.5° to 176.0° C (cor.) apparently with some decomposition as the liquid has a greenish color. The compound dissolves with difficulty at room temperature in many common organic solvents, such as alcohols and acetone, and is much less soluble in chloroform than most acetylated glycosides. $[\alpha]_D^{19} = +266$ (c, 4; $CHCl_3$). This rotation must be measured in a polarimeter because of the great difference in the tint of the two halves of the field when a saccharimeter is employed. Analysis: Calculated for $C_{20}H_{22}O_{14}N_2$: N, 5.45; Found: N, 5.50.

m-Nitrobenzyl β-D-glucoside and tetraacetate.—A modification [23] of the Koenigs-Knorr systhesis when applied to 41 g of tetraacetylglucosyl bromide and 37 g of *m*-nitrobenzyl alcohol yielded 23 g of *m*-nitrobenzyl β-glucoside tetraacetate. After three recrystallizations from ethyl alcohol, the substance melted at 120.0° to 120.5° C (cor.) and had a specific rotation $[\alpha]_D^{20} = -42.1$ (c, 4.2; $CHCl_3$). Analysis: Calculated for $C_{21}H_{25}O_{10}N$: N, 2.90; Found: N, 2.81.

Free glucoside.—Catalytic deacetylation led to the free glucoside which, after purification and subjection to a high vacuum at a temperature of 100° C, melted at 158° to 159° C (cor.). It crystallizes from methanol as squares chunky prisms. The anhydrous material rotates, $[\alpha]_D^{20} = -52.8$ (c, 2; water). These values agree with those of 157° to 158° C and −52.6 previously reported for this compound by Bourquelot and Ludwig,[24] who obtained it by enzymic synthesis. Analysis: Calculated for $C_{13}H_{17}O_8N$: N, 4.44; Found: N, 4.36.

2. OPTICAL-ROTATION MEASUREMENTS

(a) INFLUENCE OF TEMPERATURE

For the measurement of the influence of temperature on the optical rotation, a thermostated air bath (12 in. wide by 15 in. deep by 17 in. high) was constructed. The bath consisted of a wooden box lined with asbestos sheets and equipped with electric heaters (two 1,320-watt heaters in parallel) controlled by a bimetallic thermoregulator and relay. The air was kept in circulation with a motor-driven fan. The box was mounted between the nicol prisms of a Bates saccharimeter after removal of the trough. A trough was placed in the box in such position that it would hold a polariscope tube along the optic axis of the instrument. At the ends of the trough, holes were drilled in the sides of the box. Each hole was covered with two glass plates separated by an air space. (Polariscopic cover glasses were found particularly suitable, one on the inside and one on the outside of each hole in the box. They are easy to fit and are optically clear and inactive.)

[23] W. W. Pigman and N. K. Richtmyer, J. Am. Chem. Soc. **64**, 369 (1942).
[24] E. Bourquelot and A. Ludwig, Compt. rend. **158**, 1037 (1914).

The tube used to contain the solution consisted of a jacketed, 2-dm brass tube with tublature in which a thermometer could be inserted.

For the measurements, a weighed quantity of the acetylated glucoside was made up to volume at 20° with nitrobenzene. The optical rotation was measured at 20° C. The tube containing the solution then was placed in the air bath, and the rotations were read at intervals up to 150° C. At each of the temperatures selected for the measurements, the thermostatic control was placed in operation and the temperature was allowed to attain constancy. (About 30 min usually was necessary.) The temperature inside the tube and inside the box was recorded for each optical-rotation measurement. Temperature constancy of about ±1° C was achieved. After the temperature measurements at 150° C had been completed, the solution was allowed to cool and was reread to 20° C. As noted in a previous section, several of the glucosides were unstable under these conditions, and the final rotations were different from those initially measured.

In order to translate the observed optical rotations into specific rotations, it is necessary to correct for the changes in the density of the solutions with temperature. Instead of using the density of the solutions, the known density of the solvent (nitrobenzene) was employed. It is believed that these values are sufficiently close to those of the solutions that no appreciable error is introduced. The density values employed are those measured by Jaeger.[25] Calculations of the expansion of brass over the temperature range 20° to 150° C showed that the expansion of the tube was not enough to influence the results. The values obtained are recorded in table 5.

TABLE 5.—*Variation of optical rotations of acetylated glucosides in nitrobenzene solution with temperature*

Temperature	c	°S	$[\alpha]_D^t$	Temperature	c	°S	$[\alpha]_D^t$
Phenyl β-glucoside tetraacetate				*m*-Nitrobenzyl β-glucoside tetraacetate			
	g/100 ml				*g/100 ml*		
21	2.038	−2.97	−25.2	19	2.058	−5.34	−44.9
59		−2.62	−22.9	60		−4.65	−40.4
100		−2.45	−22.3	98		−4.23	−38.0
152		−2.36	−22.6	148		−3.60	−34.0
p-Nitrophenyl β-glucoside tetraacetate				*o*, *p*-Dininitrophenyl β-glucoside tetraacetate			
20	1.907	−3.80	−34.5	20	0.827	+0.84	+17.6
60		−3.43	−32.1	52		+.16	+3.4
100		−3.25	−31.6	80		−.25	−5.5
149		−3.09	−31.6	110		(−.23)	(−5.2)
				(18)		(+2.34)	(+49)
m-Nitrophenyl β-glucoside tetraacetate				*o*, *o* ¹ Dinitrophenyl β-glucoside tetraacetate			
20	2.004	−3.87	−33.4	19	2.023	+29.3	+250
61		−3.50	−31.2	51		+24.6	+215
101		−3.33	−30.8	79		+20.1	+181
150		−3.07	−29.8	110		(+10.1)	(+93)
				(20)		(+6.5)	(+56)
o-Nitrophenyl β-glucoside tetraacetate				*n*-Amyl β-glucoside tetraacetate			
20	2.013	+2.31	+19.9	21	2.071	−3.40	−28.4
51		+0.96	+8.5	62		−2.92	−25.2
79		−.06	−0.5	100		−2.61	−23.3
109		−.51	−4.7	149		−2.17	−20.4
151		−1.29	−12.5				

[25] F. M. Jaeger, Z. anorg. Chem. **101**, 112 (1917).

(b) MEASUREMENTS OF ROTATORY DISPERSION

The rotatory dispersions were measured by the use of a Schmidt & Haensch precision polariscope equipped with a monochromator. A mercury-vapor lamp (H–4; 100 w.) provided lines at 546 $m\mu$ and 436 $m\mu$. For the 436 line, the amount of light passing through the monochromator was insufficient; therefore, the light was passed through a "monochromatic" glass filter (Corning M 440). An electric sodium lamp with bichromate filter provided a light source at 589 $m\mu$. Two other lines at about 510 and 640 mμ were provided by the use of a tungsten lamp and the monochromator. For the latter lines, the wavelength was determined at each measurement by reading a quartz plate of known rotation; the reading obtained for the quartz plate was used to calculate the wavelength by reference to the known data for the dispersion of quartz.

Four-decimeter tubes were used, and the same solution was used for the rotation measurements at each of the selected wavelengths. The experiments were carried out in a room in which the temperature was controlled between 18° and 21° C. The data are given in table 6 and summarized in table 4.

TABLE 6.—*Rotatory dispersions of glucosides and tetraacetates*

Aglycon group	c	λ	$[\alpha]^{18°\text{ to }21°}$	Aglycon group	c	λ	$[\alpha]^{18°\text{ to }21°}$
β- or α-glucosides in water							
	g/100 ml	*mμ*			*g/100 ml*	*mμ*	
C_6H_5-(β)	1.62	436	−149	*m*-NO_2-C_6H_4-(β)	2.01	436	−194
		512	−102			511	−126
		546	−87			546	−107
		589	−73			589	−89
		645	−59			641	−72
p-NO_2-C_6H_4-(β)	1.61	436	−255	*o*-NO_2-C_6H_4-(β)	5.04	436	−280
		511	−151			513	−160
		546	−126			546	−130
		589	−103			589	−105
		644	−83			645	−81
m-NO_2-C_6H_4-(α)	0.99	436	+398				
		511	+267				
		546	+225				
		589	+190				
		641	+156				
β-glucoside tetraacetates in chloroform							
C_6H_5-	4.01	439	−49.6	*o, o'*-Dinitro-C_6H_3-	4.04	436	+1000
		511	−31.6			512	+458
		546	−26.6			546	+347
		589	−22.3			589	+266
		641	−18.2			644	+200
p-NO_2-C_6H_4-	4.06	439	−106	*o, p*-Dinitro-C_6H_3-	4.02	443	+166
		511	−59.6			510	+67.6
		546	−48.3			546	+48.5
		589	−39.1			589	+34.5
		641	−31.3			640	+24.0
m-NO_2-C_6H_4-	4.02	436	−85.6	*m*-NO_2-$C_6H_4CH_2$-	4.02	443	−83.4
		511	−53.6			511	−58.6
		546	−45.2			546	−49.5
		589	−36.6			589	−41.5
		644	−29.9			644	−34.0
o-NO_2-C_6H_4-	4.01	436	+230				
		512	+86.3				
		546	+60.0				
		589	+43.1				
		644	+30.3				

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